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(71) Applicant *(for all designated States except US)*: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: NAKAFUKU, Masato [JP/JP]; 4-1-1, Ogawahigashi, N102, Kodaira, Tokyo 187 (JP). KAZIRO, Yoshito [JP/JP]; 6-22-10, Shakujii-Machi, Nerimaku, Tokyo 177 (JP).

(74) Agents: LUNN, Paul, G. et al.; Schering-Plough Corporation, One Giralda Farms, M3W, Madison, NJ 07940-1000 (US).

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(57) Abstract

Methods for blocking Ras-induced conditions such as proliferative abnormalities in eukaryote, e.g., mammalian cells. Proteins and mimetics, and their uses, which can block abnormal intracellular signaling often leading to uncontrolled proliferation, e.g., cancers.

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RAS ASSOCIATED GAP PROTEINS

BACKGROUND OF THE INVENTION

Many proliferative cell abnormalities, e.g., cancers, are caused by alterations in the cellular genome. Mutations can affect the expression or function of genes controlling cell growth and differentiation. See, e.g., Bos (1989) Cancer Research 49:4682-4689. Examples of such oncogenic mutations include members of the Ras family. See, e.g., Mangues et al. (1992) Seminars in Cancer Research 3:229-239. These genes were initially studied as the viral oncogenes of several transforming retroviruses, and their relationship to cellular counterparts was soon recognized. Genes in the Ras family have been shown to be involved in the transduction of extracellular signals and the control of cellular growth.

The Ras family includes three functional genes designated H-ras, K-ras, and N-ras, which encode highly similar proteins. See Barbacid (1987) Ann. Rev. Biochem. 56:779-827. Ras genes from different human tumors were characterized and found to have undergone point mutations leading to constitutive activation, especially codons 12, 13, and 61. These mutant versions are especially potent inducers of tumorigenic or oncogenic transformation. Mutations in the Ras genes may be responsible for as many as 90% of pancreatic adenocarcinomas.

The Ras proteins are guanosine triphosphate (GTP)-binding proteins, and serve as a molecular switch in signal transduction controlling the proliferation and differentiation of cells. The linkage of Ras with the nucleoside is non-covalent and designated Ras•GXP to distinguish from a "-" which would indicate a covalent bond. Two different conformational forms of the protein exist depending upon the type of guanine nucleoside

attached to the protein. The Ras•GDP form is an inactive form which does not stimulate the downstream effector, e.g., target protein, to result in functional signal transduction. However, the Ras•GTP conformation is active, e.g., stimulates the effector to transmit an activation signal.. Interconversion between the two conformations is enzymatically effected. Conversion from the protein•GDP conformation to the protein•GTP conformation causes activation, and is described as an activation step.

Somatic mutations which constitutively activate Ras, e.g., oncogenic Ras, may contribute to tumorigenesis in up to 30% of human tumors. See, e.g., Bos (1989) Cancer Res. 49:4682- 4689; and Rodenhuis (1992) Seminars in Cancer Biol. 3:241-247. Most anti-cancer drugs currently available are not directed toward specific oncogenes, but rather inhibit even normal cellular processes. These drugs are non-specific and cause severe side-effects, e.g., killing any and all proliferating cell types. Many of these proliferating cells are important for sustaining the organism, e.g., the hematopoietic and immune systems and the intestinal lining. Treatment for proliferative cell conditions, e.g., chemo- or radio-therapy have debilitating side effects due to the nonspecificity of the drugs.

A need exists for means to more directly target therapeutic reagents to the proper abnormal cells. The next generations of anti-cancer drugs will be compounds which specifically target particular oncogenes, e.g., Ras. Thus, the development of anti-cancer drugs specifically targeting Ras oncogenes is an important goal to conquer human malignancies. The present invention provides these and many other advantages.

SUMMARY OF THE INVENTION

The present invention provides methods for blocking Ras-induced effects on eukaryotic cells. Different Ras mutations have been demonstrated to cause oncogenic transformation in eukaryotic cells by providing constitutive activation signaling to the cells. Various fragments of GTPase Activating (GAP) proteins have been identified which specifically interact with defined Ras mutants to block signal transduction. These fragments likely function through a mechanism of interacting with the Ras•GTP activated conformation to block the natural interaction of the effector protein. These fragments thus block the constitutive signal transduction which results in Ras induced constitutive effects.

The present invention provides methods of blocking a Ras-induced effect on a cell, comprising a step of introducing a GTPase Activating (GAP) protein to the cell. Ordinarily, the Ras will be an oncogenic Ras or one which substantially lacks GTPase activity. The Ras-induced effect will typically be induction of cell proliferation or transformation. The cell will often be eukaryotic cell, e.g., a mammalian cell, including a human cell. On some embodiments, the step of introducing is by expression of a nucleic acid encoding the GAP protein.

In preferred embodiments, the GAP protein will bind to the Ras protein with a Kd of less than 200 nM. In other embodiments, the GAP protein is selected from: (a) a fragment of a mammalian GAP protein; (b) a fragment of a mammalian NF1-GRD protein; and (c) a homologue or mimetic of (a) or (b). In particular embodiments, the GAP protein is selected from: (a) a fragment of a mammalian GAP protein having a wild type sequence, including a human GAP protein; and (b) a fragment of a mutant mammalian GAP protein having a sequence with an amino acid substitution at a position corresponding to a position 1063 through 1651 of NF1 or the

corresponding region of other GAP proteins. Many of these substitutions will be a conservative substitution.

In other embodiments, the GAP protein will interact with Ras and block interaction of an effector molecule which binds to Ras at a position corresponding to a position from 32 to 40 or from 59 to 65.

In various preferred embodiments, the GAP protein does not block signal transduction of non-oncogenic Ras. Greater specificity of action can be achieved by identifying the responsible oncogenic Ras and selecting a GAP protein which specifically blocks the identified oncogenic Ras.

The invention also provides methods of treating an oncogenic Ras transformed cell comprising the step of introducing to said cell a GAP protein capable of suppressing the transformation of said cell. Often, the oncogenic Ras transformed cell will be a mammalian cell, including a human cell.

In some embodiments, the GAP protein does not block signal transduction of non-oncogenic Ras. The method can be improved by adding steps of identifying the responsible oncogenic Ras and selecting a GAP protein which blocks transformation by the identified Ras. Preferably, the GAP protein does not block signal transduction of non-oncogenic Ras, e.g., exhibiting specificity.

In addition, the invention provides methods of identifying appropriate GAP proteins useful for treating a mutated Ras-induced condition of a eukaryote cell comprising: (a) identifying the mutated Ras which induces the condition; and (b) screening various GAP variants for specific variants which are capable of blocking the condition. In some embodiments, the eukaryote cell is a mammalian cell, including a human cell. In a preferred embodiment, additional screening is performed to determine which GAP variants have minimal effect on non-mutated Ras effects.

The invention further provides GAP proteins capable of blocking transformation of a cell, where said

transformation is due to an oncogenic Ras. In some cases, the GAP protein is selected from: (a) a fragment of a mammalian GAP protein; (b) a fragment of a mammalian NF1-GRD protein; and (c) a homologue or mimetic of (a) or (b). In others, the GAP protein is selected from: (a) a fragment of a mammalian GAP protein having a wild type sequence, including a human GAP protein; and (b) a fragment of a mutant mammalian GAP protein having a sequence with an amino acid substitution at a position corresponding to a position from 1063 through 1651 of NF1 or the corresponding region of other GAP proteins. Often the substitution will be a conservative substitution. In other embodiments, the protein interacts with Ras and blocks interaction of an effector molecule which binds to Ras at a position from 32 to 40 or from 59 to 65. Often the cell is a eukaryotic cell, e.g., a mammalian cell, including a human cell.

In preferred embodiments, the oncogenic Ras substantially lacks GTPase activity. In other embodiments, the protein binds to oncogenic Ras with a Kd of less than 200 nM. Mechanistically, the protein may interfere with interaction of Ras•GTP with an effector compound. In another embodiment, the invention provides an isolated nucleic acid encoding a protein normally expressed as a protein as described.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows stimulation of GTPase activity of c-Ha-Ras^{Gly12} and c-Ha-Ras^{Val12} proteins by yeast cell extracts containing wild-type and mutant NF1-GRDs.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

OUTLINE

I. Ras family
   A. structure and function
   B. cycling between Ras•GDP and Ras•GTP
II. GAP proteins; family, mammalian, NF1
III. Interaction of Ras and GAP proteins
IV. Downstream signal transduction
V. Methods
   A. administering
   B. matching to corresponding Ras
   C. making compositions, analogues, mimetics

I. Ras family

A. structure and function

Ras gene family members are ubiquitous among eukaryotic cells. See, e.g., Barbacid (1987) Ann. Rev. Biochem. 56:779-827. The genes were initially identified and studied as the viral oncogenes of several acute transforming retroviruses. The relationship to human cancer was quickly established upon recognition that the retroviral oncogenes were derived from a group of mammalian cellular proto-oncogenes, e.g., endogenous genes which become oncogenic upon mutation.

Point mutations in the normal endogenous mammalian Ras gene often led to an oncogenic transformed phenotype. Further studies on the locations of the point mutations showed a high frequency at particular hot spots, e.g., codons 12, 13, or 61. Recent technology, e.g., selective hybridization with specific probes, and PCR techniques have simplified analysis of specific alterations responsible for

Ras-induced effects. See Bos (1988) Mutat. Res. 195:255-271.

Extension of interest to the counterparts in non-mammalian systems has shown that these genes play a critical role in transduction of many extracellular signals in cells. Functional and structural data has shown that Ras proteins are GTP-binding proteins involved in transduction of signals in response to extracellular stimuli. The family of Ras proteins can be defined by a combination of functional and structural criteria. See, e.g., Bollag et al. (1991) Ann. Rev. Cell Biol. 7:601-632. Ras-induced effects are the functional consequences of Ras activation.

In mammalian cells, typically the Ras-induced effects will be cell transformation, but may also include differentiation or proliferation effects which fail to satisfy the full criteria for transformation.

The yeast Saccharomyces cerevisiae possesses two members of the Ras family (Ras1 and Ras2) which play an important role in cell growth through the regulation of adenylate cyclase. See, e.g., Broach et al. (1990) Adv. Cancer Res. 54:79-139. The Ras-induced effects in yeast show a heat-shock sensitive phenotype.

Members of the Ras family have also been studied in Xenopus laevis; Drosophila melanogaster, Caenorhabditis elegans; and Dictyostelium discoideum. See Bollag et al. (1991) Ann. Rev. Cell Biol. 7:601-632; and Kaziro et al. (1991) Ann. Rev. Biochem. 60:349-400.

Although the Ras-induced effects may be different in different cells, the relationship in structure often allows cross species interactions of corresponding proteins in Ras related pathways. Exploitation of these structural similarities provide useful means to test interaction of proteins which normally are never found together with advantages directed towards ease of testing effects on various cell sources.

B. cycling between Ras•GDP and Ras•GTP

The Ras proteins have been shown to be GTP-binding proteins. They can be either in GDP-bound conformation or a GTP-bound conformation. The GTP-bound conformation is the active and interacts with an as yet unidentified effector molecule.

Current models propose that Ras proteins become activated upon stimulation, transduce the signal to an as yet unidentified effector molecule, and subsequently become inactivated. Mutated, e.g., oncogenic, Ras proteins have lost their ability to become inactivated and thus constitutively send a stimulation signal.

Ras is active in its GTP-bound form. The active Ras•GTP complex, which is a non-covalent association, is converted to an inactive Ras-guanosine diphosphate (Ras•GDP) form by an intrinsic GTPase activity found on normal Ras, and which is stimulated by a GTPase Activating (GAP) protein. However, oncogenic Ras lacks the intrinsic GTPase activity and GAP proteins have little, if any, effect on inactivating oncogenic Ras. This substantial lack of GTPase activity in oncogenic Ras will typically be at least 20% less than the normal, more typically at least 35% less, usually at least 50%, more usually at least 60% less, preferably at least 70% less, and more preferably at least 80% or more less than normal Ras.

II. GAP proteins; family, mammalian, NF1

GTPase activities are required to inactivate the Ras•GTP form of the protein in the cycling reaction. A family of proteins stimulating endogenous GTPase activities of Ras proteins have been described which share structural and functional similarities. See Bollag et al. (1991) Ann. Rev. Cell Biol. 7:601-632. Particularly relevant members of the GAP family include yeast and mammalian proteins, including the human neurofibromatosis type 1 (NF1) protein. As used herein, GAP protein refers to a protein which shares structural or functional properties with this family of proteins. Usually, the protein will be a fragment

shorter than the natural mammalian proteins so far described, normally less than about 600 amino acids, more normally less than about 550 amino acids, ordinarily less than about 500 amino acids, more ordinarily less than about 460 amino acids, usually less than about 420 amino acids, more usually less than about 380 amino acids, typically less than about 350 amino acids, more typically less than about 325 amino acids, preferably less than about 310 amino acids, more preferably less than about 300 amino acids, and in other embodiments, even fewer amino acids, down to 200 or fewer amino acids.

NF1 was first identified as the gene responsible for the pathogenesis of the human genetic disorder, neurofibromatosis type 1. cDNA cloning revealed that the NF1 gene encodes a protein of 2818 amino acids. This putative protein product has a domain showing a significant sequence homology with members of the Ras GTPase-activating protein (GAP) family. See, e.g., Gutmann et al. (1992) Ann. Neurol. 31:555-561; Xu et al. (1990) Cell 63:835-841; Martin et al. (1990) Cell 63:843-849; and Ballester et al. (1990) Cell 63:851-859. This domain, a fragment of the natural NF1, is often referred herein as NF1 GAP Related Domain (NF1-GRD), and some fragments thereof should have similar activities.

Two yeast Saccharomyces cerevisiae proteins, Ira1 and Ira2, show particularly high sequence homology to the NF1. Subsequent studies have demonstrated that members of the GAP family, including the GAP-related domain of the NF1 gene product (NF1-GRD; sometimes referred to as NF1 fragment), can stimulate guanosine triphosphatase (GTPase) activity of Ras proteins, i.e., converting Ras•GTP to Ras•GDP, and thereby negatively regulate the activity of Ras.

Two proteins which regulate the activity of Ras proteins are the GTPase activating protein (GAP) and the protein encoded by NF1, the gene responsible for neurofibomatosis. type I disease. See Gutmann et al. (1992) Ann. Neurol. 31:555-561.

III. Interaction of Ras and GAP proteins

The GAP proteins have been identified as one of the means by which activated Ras proteins are converted into the inactive form. Thus, the physical interaction of the GAP and Ras proteins are important in the understanding of the functional relationship between the entities.

The GAP protein effect on endogenous GTPase activity of RAS has been localized to a fragment of the natural GAP protein, e.g., wild-type sequences. In particular, the catalytic domain has been localized to the carboxy terminal segment of the mammalian GAP proteins. The active portion has been localized to a fragments of less than about 600 amino acids, corresponding to the NF1 amino acids 1063-1651. As such, the functional activities of the GAP proteins would be expected to be localized in this region of the sequence. The sites of GAP interaction with Ras have been proposed to be positions 32-40 and 59-63 of mammalian Ras.

The yeast S. cerevisiae possesses two NF1 homologues, Ira1 and Ira2. The human NF1 is structurally closer to yeast Ira than human GAP and thus would be expected to interact well with the yeast Ras counterpart proteins. This structural similarity is reflected in a functional relationship, as NF1-GRD expressed in yeast cells can complement ira-deficient yeast. In ira$^-$ cells, the conversion of Ras•GTP to Ras•GDP is defective, and the cells show a phenotype which is very similar to that of activated Ras mutants, i.e., heat shock-sensitivity. The GAP-Related Domain of the NF-1 gene product (NF1-GRD) is a fragment from the NF-1 which can suppress the heat-sensitive phenotype of ira$^-$, but not of RAS2^{Val19} or RAS2^{Leu68}. This is consistent with the fact that NF1-GRD stimulates GTPase activity of normal but not mutant Ras proteins. Thus, the natural GAP will have blocking effects of Ras functions of normal cells.

IV. Downstream signal transduction

The biochemical mechanism of signal transduction, or effect, of Ras activation is poorly understood. The structural means by which signal transduction occurs has not been clarified, but it is believed that an effector compound, likely a protein, interacts with Ras•GTP. Genetic analysis of the amino acid positions which affect effector binding have been postulated to include positions 32, 35, 36, 38 and 40. Thus, the effector may well bind near to the same sites of Ras as does the GAP proteins.

This has led to the model that variants of GAP segments may interact with Ras in a fashion which can block effector interaction. This will function to block signal transduction, in a fashion which will inactivate an oncogenically transformed Ras. Moreover, since the different oncogenic Ras forms result from mutations at sites near the GAP and effector interaction sites, variant GAP segments may show great specificity in blocking Ras-induced effects. In particular, the binding affinity of the GAP analogues which block Ras-induced effects are higher than normal GAP binding.

In particular embodiments, the GAP protein, which is intended here to also encompass the concept of protein analogues and mimetics, will preferably be a relatively small polypeptide or analogue, including modified proteins and mimetics. Mimetics include compounds possessing similar molecular shapes sufficient to confer the desired biological property. Various amino acid substitutions may be designed, tested, or screened for activity in blocking Ras-induced functions. These may be effective in blocking effects of many different Ras mutants, or specific Ras variants. The methodology described herein may be useful to define GAP proteins which exhibit high specificity for only interacting with oncogenic, e.g., mutant Ras, and having virtually no effect on natural Ras function. Thus, the GAP proteins provided herein will be highly specific in affecting only oncogenic functions and will be innocuous in cells possessing normal Ras.

Although the positions of GAP believed to be most important in the interaction with Ras are in the regions of 701-1047 of GAP, the NF1 regions considered most likely to be useful herein will be within the region of 1063-1651 or the corresponding region of other GAP proteins, including 1175-1534, and more specifically in the regions of 1400-1500. Mutations within this region are likely to interact with the Ras in the desired way, particularly in the region of 1421-1461 of NF1 or the corresponding region of other GAP proteins.

Functionally, the useful GAP proteins have high binding affinity for Ras or Ras-like proteins or GAP binding segments thereof. Typically, the GAP protein will exhibit a Kd for Ras, or its oncogenic variant, of less than about 300 nM, more typically less than about 250 nM, usually less than about 200 nM, more usually less than about 150 nM, preferably less than about 100 nM, and more preferably even higher binding affinity. Typically a higher binding affinity will allow effective competitive effect on the effector binding at low concentrations of GAP protein.

IV. Methods

A. administering

As described, blocking Ras-induced effects will occur upon proper selection of the GAP protein, e.g., fragments, analogues, and mimetics, and administering such composition to the cell. The GAP protein will be produced, e.g., by recombinant means, as are described in Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual Cold Spring Harbor Press, CSH, N.Y., and Ausubel (1987 and periodic supplements) Current Protocols in Molecular Biology Greene/Wiley, New York; which are each incorporated herein by reference. The GAP protein can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, along with physiologically innocuous

stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations.

Drug screening using Ras or fragments thereof can be performed to identify compounds having binding affinity. Subsequent biological assays can then be utilized to determine if the compound has intrinsic activity and is therefore a blocker or antagonist in that it blocks the effects of oncogenic Ras. Additional compounds may be screened or designed using the reagents described, or by molecular modeling and structural studies including, e.g., X-ray crystallography, multidimensional NMR, and other techniques. See, e.g., Blundell et al. (1976) Protein Crystallography Academic Press, New York.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman et al. (eds) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; each of which is hereby incorporated herein by reference. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Dosage ranges would ordinarily be expected to be in amounts lower

than 100 mM concentrations, typically less than about 10 mM concentrations, usually less than about 100 μM, preferably less than about 10 μM, and most preferably less than about 1 μM, with an appropriate carrier. Slow release formulations, or slow release apparatus will often be utilized for continuous administration.

The GAP protein may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier must be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous, and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman et al. (eds) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; each of which is hereby incorporated herein by reference. The therapy of this invention may be combined with or used in association with other chemotherapeutic or chemopreventive agents.

Isolation and characterization of these nucleic acids allow use thereof to make variants and mutants. It will also allow production of vector constructs useful, e.g., for gene therapy. See, e.g., Goodnow (1992) "Transgenic Animals" in Roitt (ed.) Encyclopedia of Immunology Academic Press, San Diego, pp. 1502-1504; Travis (1992) Science

256:1392-1394; Kuhn et al. (1991) Science 254:707-710; Capecchi (1989) Science 244:1288; Robertson (1987)(ed.) Teratocarcinomas and Embryonic Stem Cells: A Practical Approach IRL Press, Oxford; and Rosenberg (1992) J. Clinical Oncology 10:180-199; which are each incorporated herein by reference.

B. matching to corresponding Ras

In particular, the present invention allows for simple matching of a therapeutic agent to various oncogenic Ras variants. This can provide highly selective treatment of defined oncogenic conditions with a GAP having highly selected safety and efficacy combinations, virtually tailored to the relatively small number of oncogenic Ras mutations which cause defined proliferative conditions. For example, common variants of oncogenic Ras can be used to screen for GAP fragments which are effective in blocking the oncogenic effects. See, e.g. Kumar et al. (1990) Cancer Res. 52:6877-6884. Either the variants or equivalents thereof can be transformed into a cell, e.g., a yeast cell, and GAP mutants tested for their specific effect on the Ras variants. Once appropriate GAP proteins are identified for each of the common oncogenic Ras mutants, therapeutic reagents can be selected based upon the diagnosed mutant oncogenic Ras responsible for a given abnormality. Diagnosis of the responsible Ras mutation can be performed as described above.

C. making compositions, analogues, mimetics

Isolated GAP encoding DNAs can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode these modified GAP proteins, their derivatives, or proteins having the desired anti-oncogenic activity. These modified sequences can be used to produce mutant GAP proteins or to enhance the expression of GAP. Enhanced expression may involve gene

amplification, increased transcription, increased.... translation, and other mechanisms. Such mutant Ras or GAP derivatives include predetermined or site-specific mutations of the respective protein or its fragments. A mutant GAP is a polypeptide otherwise falling within the homology defined by structure and function, but having an amino acid sequence which differs from the corresponding segment of GAP as found in nature, whether by way of an amino acid deletion, substitution, or insertion. Similar proteins and nucleic acids should be available from other warm blooded animals, e.g., mammals and birds. These descriptions are generally meant to encompass species and allelic variants of the GAP proteins, not limited to the specific embodiments discussed.

Although site specific mutation sites are predetermined, mutants need not be site specific. GAP protein or Ras protein mutagenesis can be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include but are not limited to amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed GAP mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis. See also Sambrook et al. (1989) and Ausubel et al. (1987 and Supplements).

The mutations in the DNA normally should not place coding sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these proteins. A heterologous fusion

protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of an immunoglobulin with a GAP polypeptide is a continuous protein molecule having sequences fused in a typical peptide linkage, e.g., typically made as a single translation product and exhibiting properties derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, Ras-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham et al. (1989) Science 243:1330-1336; and O'Dowd et al. (1988) J. Biol. Chem. 263:15985-15992, each of which is incorporated herein by reference. Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of Ras-binding specificities. For example, the Ras-binding segments from other related proteins may be added or combined with other binding segments from other proteins. The resulting protein will often have hybrid function and properties.

The phosphoramidite method described by Beaucage and Caruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

The present invention provides means to produce fusion proteins. Various GAP variants may have slightly different functions or biological activities, even though they share significant structural similarities. Dissection of structural elements which effect the various physiological functions or biological activities

provided by the GAP proteins is possible using standard techniques of modern molecular biology, particularly in comparing variants within the related family of GAP proteins. See, e.g., the homolog-scanning mutagenesis technique described in Cunningham et al. (1989) Science 243:1339-1336; and approaches used in O'Dowd et al. (1988) J. Biol. Chem. 263:15985-15992; and Lechleiter et al. (1990) EMBO J. 9:4381-4390; each of which is incorporated herein by reference.

In particular, Ras binding segments can be substituted between proteins to determine what structural features are important in both Ras binding affinity and specificity for the natural or oncogenic Ras. An array of different Ras variants, e.g., allelic, will be used to screen for GAP proteins exhibiting desired properties of interaction with them, e.g., high binding affinity, blocking of effector function by conformational or competitive inhibition, or even forms which can induce GTPase action of the oncogenic Ras. The specific segments of interaction of GAP with Ras may be identified by mutagenesis or direct biochemical means, e.g., cross-linking or affinity methods. Structural analysis by crystallographic or other physical methods will also be applicable. Identification of the similarities and differences between Ras oncogenic variants will lead to new diagnostic and therapeutic reagents or treatments.

Structural studies of the Ras variants will lead to design of new GAP proteins, particularly analogues exhibiting desired effect blocking properties. This can be combined with screening methods to isolate new GAP proteins exhibiting desired spectra of activities. Both the naturally occurring and the recombinant forms of Ras are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to them. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds per year. See, e.g.,

Fodor et al. (1991) Science 251:767-773, which is incorporated herein by reference and which describes means for testing of binding affinity by a plurality of defined polymers synthesized on a solid substrate. Phage or other libraries of various random polypeptide sequences would also be useful. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble Ras, either natural or oncogenic, by methods as provided herein.

Expression in other cell types will often result in glycosylation differences in a particular GAP protein. Various mutants may exhibit distinct biological activities based upon structural differences other than amino acid sequence. Differential modifications may be responsible for differential function, and elucidation of the effects are now made possible.

A nucleic acid which encodes a Ras and GAP are readily available, or can be obtained by chemical synthesis, screening cDNA libraries, or by screening genomic libraries prepared from a wide variety of cell lines or tissue samples. See, e.g., Marchuk et al. (1991) Genomics 11:931-940; and nucleic acid and protein data bases, e.g., Protein Identification Resource (PIR), Georgetown University, Washington, D.C., SwissProt and others, see IntelliGenetics, Menlo Park, CA, or the Univ. Wisconsin Biotechnology Center, Madison, Wisconsin.

This DNA can be expressed in a wide variety of host cells for the synthesis of a Ras, GAP. or fragments thereof which can in turn, for example, be used to generate polyclonal or monoclonal antibodies; for construction and expression of modified Ras or GAP molecules; and for structure/function studies. Each GAP can be expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially free of protein or cellular contaminants, other than those derived from the

recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The GAP, or portions thereof, may be expressed as fusions with other proteins.

Expression vectors are typically self-replicating DNA or RNA constructs containing the desired Ras or GAP gene or its fragments, usually operably linked to suitable genetic control elements that are recognized in a suitable host cell. These control elements are capable of effecting expression within a suitable host. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription and translation. Expression vectors also usually contain an origin of replication that allows the vector to replicate independently of the host cell.

The vectors of this invention contain DNA which encodes a useful GAP-like peptide, or a fragment thereof encoding, e.g., an active polypeptide. The DNA can be under the control of a viral promoter and can encode a selection marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNA coding for a GAP in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNA coding for the GAP is inserted into the vector such that growth of the host containing the vector expresses the cDNA in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number

of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate in a host cell, e.g., it is possible to effect transient expression of the GAP in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of GAP into the host DNA by recombination.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector but all other forms of vectors which serve an equivalent function and which are, or become, known in the art are suitable for use herein. See, e.g., Pouwels et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., and Rodriquez et al. (eds) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Buttersworth, Boston, 1988, which are incorporated herein by reference.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., E. coli and B. subtilis. Lower eukaryotes include yeasts, e.g., S. cerevisiae and Pichia, and species of the genus Dictyostelium. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, E. coli and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for amplifying DNA is pBR322 or many of its derivatives. Vectors that can be used to express the GAP protein include, but are not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius et al. (1988) "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters", in Vectors: A Survey of Molecular Cloning Vectors and Their Uses, (eds. Rodriguez and Denhardt), Buttersworth, Boston, Chapter 10, pp. 205-236, which is incorporated herein by reference.

Lower eukaryotes, e.g., yeasts and Dictyostelium, may be transformed with GAP sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, Saccharomyces cerevisiae. It will be used to generically represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the Ras or GAP protein or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression

vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionine promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), or mini-chromosomes (such as the YCp-series).

Higher eukaryotic cells grown in tissue culture are often the preferred host cells for expression of the GAP protein. In principle, any higher eukaryotic tissue culture cell line is workable, e.g., insect baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are often preferred. Transformation or transfection and propagation of such cells has become a routine procedure. Examples of useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for such cell lines usually include an origin of replication, a promoter, a translation initiation site, RNA splice sites (if genomic DNA is used), a polyadenylation site, and a transcription termination site. These vectors also usually contain a selection gene or amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1 (Invitrogen, San Diego, CA); pCD, see Okayama et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo Poly-A, see Thomas et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610.

It may be desired to express a GAP polypeptide in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system. However, the pattern will be modifiable by exposing the polypeptide, e.g., an unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, the GAP gene may be co-transformed with one or more genes encoding mammalian or other glycosylating enzymes. Using this approach, certain mammalian glycosylation patterns will be achievable in prokaryote or other cells.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the invention in any manner.

EXAMPLES

In these studies, a yeast Ras system was used to isolate NF1-GRD mutants which can suppress specifically the activity of oncogenic Ras. Yeast cells carrying activated mutations in Ras (such as RAS2^{Val19} and RAS2^{Leu68}) are defective in responding to environmental conditions, and show a variety of phenotypes including a heat shock-sensitive phenotype.

First, a pool of randomly mutagenized NF1-GRD genes were screened to obtain suppressors of a specific yeast oncogenic-type Ras, RAS2^{Val19}. Next, these mutant NF1-GRDs were shown to be capable of inhibiting v-Ras-induced transformation in mammalian cells. These results demonstrated that this unique yeast method provides a powerful screening system to obtain anti-Ras NF1-GRD mutants. The mutants of NF1-GRD most likely bind tightly with the oncogenic, e.g., mutated, Ras proteins to sequester the latter proteins from the signal transduction for normal cell growth. Detailed analysis of the

structures involved in the interaction between mutant NF1-GRDs and Ras will enable testing of compounds, e.g., analogues and mimetics, which can mimic the action of NF1-GRDs, and inhibit specifically transforming Ras activity.

EXAMPLE 1: Preparation of pKP11

A plasmid pKP11, which expresses a domain of NF1 (amino acid residues 1063-1651; the numbers of amino acid residues are referred to according to Marchuk et al. (1991) Genomics 11:931-940, and a yeast strain carrying $RAS2^{Val19}$ mutation were used to obtain mutant NF1-GAP Related Domains (GRDs) which can suppress the phenotype of activated Ras. In a previous study, this plasmid was shown to suppress $ira2^-$ but not $RAS2^{Val19}$. The plasmid was randomly mutagenized by treatment with hydroxylamine in vitro, and a pool of mutagenized DNAs was transformed into $RAS2^{Val19}$ cells. Subsequently, about 2×10^5 independent colonies were screened for heat shock resistance.

Wild-type NF1-GRD was cloned into the yeast expression vector pKT10 which contains glyceraldehyde-3-phosphate dehydrogenese promoter, a replication origin derived from 2 μm, and URA3 as a selection marker to yield pKP11. One hundred micrograms of pKP11 DNA was mutagenized by hydroxylamine in vitro as described previously (Rose et al. (1987) Cell 48:1047-1060), and transformed into a S. cerevisiae strain, TK161-R2V-D which carries $RAS2^{Val19}$ mutation. See Tanaka et al. (1989) Mol. Cell. Biol. 9:757-768; and Tanaka et al. (1990) Mol. Cell. Biol. 10:4303-4313. About 2×10^5 colonies were grown on selection plates, and the plates were heated at 57 °C for 15 minutes. The resultant plates were incubated at 30 °C for 4 days, and growing colonies were selected. The heat shock-sensitivity of these colonies were checked, and 12 clones were selected at this stage. Plasmid DNAs were recovered from these cells, re-transformed into TK161-R2V-D, and phenotypic reversion was examined.

Twelve positive colonies were obtained in the initial screening. Subsequently, two clones, NF201 (SEQ ID NO: 1) and NF204 (SEQ ID NO: 2), which had a relatively strong suppression activity for RAS2^{Val19}, were selected, and subjected to further analysis.

EXAMPLE 2: Effect of Mutant NF1-GRDs on yeast cells

The effects of NF201 (SEQ ID NO: 1) and NF204 (SEQ ID NO: 2) were tested on different alleles of activated RAS2^{Val19} in yeast cells (Table 1). Wild-type NF1-GRD could weakly revert the phenotype of RAS2^{Leu68}, but was totally ineffective on RAS2^{Val19} and RAS2^{Ser41}. Mutant NF201 suppressed the heat shock-sensitive phenotype of all three alleles of RAS2 examined, including RAS2^{Val19}, RAS2^{Leu68}, and RAS2^{Ser41} (Tanaka et al. (1992) Mol. Cell. Biol. 21:631-637). On the other hand, NF204 preferentially suppressed RAS2^{Val19} but not the other two alleles. These results indicate that NF201 and NF204 possess distinct properties as suppressors of activated Ras in a Ras-specific manner.

Table 1. Suppression of the heat-sensitive phenotypes of various activated alleles of RAS2 by mutant NF1-GRD. A wild-type S. cerevisiae strain, RAY-3A-D, harboring a combination of RAS2 plasmids (YCp-$RAS2^{Val19}$, -$RAS2^{Leu68}$, and -$RAS2^{Ser41}$; Tanaka et al. (1992) Mol. Cell. Biol. 12:631-637) and NF1-GRD plasmids, was subjected to heat shock assay. The ability of each NF1-GRD plasmid to suppress the heat-sensitive phenotype was scored: +++, strong suppression; ++, intermediate suppression; +, weak suppression; -, no detectable suppression. The complementation activity in ira2$^-$ cells (KT63-2B-D; Tanaka et al. (1989) Mol. Cell. Biol. 9:757-768; Tanaka et al. (1990) Mol. Cell. Biol. 10:4303-4313), which reflects the activity of these NF1-GRDs on wild-type RAS2 ($RAS2^{wt}$), was also scored, and is shown in the table.

	RAS2 allele			
NF1-GRD	$RAS2^{Val19}$	$RAS2^{Leu68}$	$RAS2^{Ser41}$	$RAS2^{wt}$
NF201	+++	+++	++	+++
NF204	+++	+	-	+++
NF1 (wild-type)	-	+	-	+++

Interestingly, these two mutant NF1-GRDs could suppress ira2$^-$ cells, in which normal Ras proteins are activated, to the same extent as wild-type NF1-GRD, suggesting that NF201 and NF204 retain the ability to stimulate GTPase activity of normal Ras.

The entire region of mutant NF1-GRDs were sequenced to identify mutations in NF201 and NF204, and the sequences compared the sequences with that of wild-type NF1-GRD. In both NF201 and NF204, single nucleotide changes were found in the DNA sequences. In NF201 (SEQ ID NO: 1), the codon TTC for Phe at residue 1434 was changed to TTA coding for

Leu, while in NF204 (SEQ ID NO: 2), the codon AAG for Lys at residue 1436 was replaced by AGA coding for Arg.

Although both mutation sites are located in one of the most conserved regions of the GAP-related domain (see Xu et al. (1990) Cell 63:835-842; Martin et al.(1990) Cell 63:843-850; and Ballester et al. (1990) Cell 63:851-859), the amino acid residues at these sites (Phe at position 1434, and Lys at position 1436) are not strictly conserved among the members of the GAP family (Figure 1). Phe residue at 1434 in NF1 is conserved in yeast Ira2 (SEQ ID NO: 4) protein, but it is replaced by other residues in Ira1 (SEQ ID NO: 3), GAP (SEQ ID NO: 5), and Gap1 (SEQ ID NO: 6). On the other hand, Lys residue at 1346 is conserved among NF1, Ira1, GAP, and Gap1, but Ira2 contains Arg at the corresponding site. Recently, two independent studies have demonstrated that Lys at position 1423 in NF1-GRD, which is located just 11 and 13 amino acids upstream of the mutation sites of NF201 and NF204, respectively, is important for the structure and function of NF1. First, the substitution of Glu for Lys at position 1423 has been identified in some human tumors as well as in a family of neurofibromatosis patients (Li et al. (1992) Cell 69:275-281). The GAP activity of this mutant NF1-GRD was 200- to 400-fold lower than that of the wild-type NF1-GRD. It was also reported that the substitution of Met for Lys at the same position resulted in a decrease in stability of the protein (Wiesmuller et al. (1992) J. Biol. Chem. 267:10207-10219). Thus, the amino acid residues at 1423, 1434 and 1436, and their surrounding sequence, are likely to be important for the structure and/or function of NF1 proteins.

EXAMPLE 3: Effect of mutant NF1-GRDs in mammalian cells

The effect of these mutant NF1-GRDs in mammalian cells was investigated. The cDNA fragments of the wild-type and mutant NF1-GRDs were recloned into a mammalian expression vector, and transfected into cell lines.

The size of the NF1-GRD protein transiently expressed in Cos7 cells was checked. Western blot analysis with an anti-NF1-GRD anti-serum (see Hattori et al. (1992) Oncogene 7:481-485) identified a protein band of an apparent molecular mass of 67-68 kDa in the cells transfected with NF1-GRD plasmids but not with the control vector. This suggests that the protein of about 67 kDa was translated starting from the internal Met residue at position 1073 of NF1 cDNA.

The anti-Ras activities of mutant NF1-GRDs were examined for their effects on v-Ras-induced transformation. The above plasmids expressing NF1-GRD were cotransfected with pSV2neo into DT cells, a v-Ki-ras-transformed NIH3T3 derivative, and the ability to induce morphological reversion of the cells was examined. As shown in Table 2, transfection of the plasmids expressing NF201 and NF204 could induce flat reversion at dramatically high frequencies (8-9% of total G418-resistant colonies). The frequency was even higher than that obtained by transfection of a Krev-1 plasmid which has been shown to possess anti-oncogenic activity in DT cells (Kitamura et al. (1990) Proc. Natl Acad. Sci. USA 87:4284-4288). Under the same conditions, the wild-type NF1-GRD could also induce flat reversion of DT cells, although it was 5 to 6 times less potent than mutant clones. This is particularly interesting since a previous study has shown that overexpression of GAP inhibited normal c-Ha-Ras- but not v-Ha-Ras-induced transformation (see Zhang et al. (1990) Nature 346:754-756).

No revertant of DT cells could be obtained from transfectants of the GAP plasmid (Table 2). This difference may be due to the fact that NF1-GRD possesses a much higher affinity for Ras proteins than GAP. These results clearly demonstrate that mutant NF1-GRDs possess transformation-suppressor activity against oncogenic Ras.

Table 2. Induction of morphological reversion of v-Ras-transformed cells by mutant NF1-GRD. DT cells were cotransfected with 20 μg of NF1-GRD plasmids and 2 μg of pSV2neo as described by Kitamura et al. (1990) Proc. Natl Acad. Sci. USA 87:4284-4288, and transfectants were selected in a medium containing 0.5 mg/ml G418. Since pKrev-1 plasmid itself contained the neo gene, 2 μg of the plasmid was cotransfected with 20 μg of pEF-BOS (the vector for NF1-GRD). The pEF-GAP contained rat full-length GAP cDNA in pEF-BOS. Frequency of reversion in DT cells is defined as the ratio (%) of morphologically flat cell colonies to total G418-resistant colonies. N.D.: not determined.

transfected DNA	Flat colonies/G418-resistant colonies Exp.1	Exp.2	Exp.3	ratio (%)
pEF-BOS	0/1155 (<0.1)	2/1279 (0.1)	3/878 (0.4)	0.1
pEF-NF1	20/1522 (1.3)	26/1151 (2.3)	15/1004 (1.5)	1.7
pEF-NF201	86/1190 (7.2)	61/691 (8.8)	34/356 (9.6)	8.0
pEF-NF204	40/448 (8.9)	46/426 (10.8)	24/350 (6.9)	9.0
pEF-GAP	N.D.	0/856 (<0.1)	0/561 (<0.2)	<0.1
pKrev-1	N.D.	26/1385 (1.9)	15/736 (2.0)	1.9

EXAMPLE 4: Biochemical properties of the mutant NF1-GRDs

The biological properties of the mutant NF1-GRDs were studied to understand the molecular mechanism of anti-oncogenic activity. Extracts were prepared from yeast cells expressing wild-type and mutant NF1-GRDs, and GTPase-stimulating activity was measured in vitro by using recombinant c-Ha-Ras proteins as substrates. Recombinant c-Ha-Ras^{Gly12} (A) or c-Ha-Ras^{Val12} (B) proteins were loaded with [γ-^{32}P]GTP (30 Ci/mmol) in buffer B (50 mM tris-HCl [pH 7.4], 50 mM KCl, 1 mM $MgCl_2$, 2.5 mM EDTA, and 0.2 mg/ml BSA) at 30 °C for 10 minutes. The reaction was stopped by the addition of $MgCl_2$ to the final concentration of 7 mM. Yeast cell extracts were prepared from wild-type yeast cells, RAY-3A-D, carrying various NF1-GRD plasmids. Cells grown to the stationary phase were collected, and disrupted with acid-washed glass beads (0.5 mm diameter) in buffer A (50 mM tris-HCl [pH 7.4], 100 mM KCl, 5 mM $MgCl_2$, 2 mM DTT, 2 mM PMSF, 1 mM benzamidine, and 10 µg/ml of each of pepstatin A, aprotinin, and leupeptin. The crude extract was clarified twice by centrifugation at 2000 x g for 20 minutes. The resultant supernatants were then mixed with an aliquot of Ras•[γ-^{32}P]GTP mixture, and incubated at 30 °C. At the indicated time point, an aliquot was filtered through a nitrocellulose membrane, and radioactivity retained on the membrane was counted. The final concentrations of yeast extract proteins and Ras•[γ-^{32}P]GTP were 1 mg/ml and 11.5 nM, respectively. The cell extracts assayed were from the cell carrying the following plasmids: ●, wild-type NF1-GRD; o, NF201; Δ, NF204; [solid square], vector alone; or [open square], buffer A plus 1 mg/ml BSA. Two mutant NF1-GRDs, NF201 and NF204, stimulated the GTPase activity of c-Ha-Ras^{Gly12} to the same extent as wild-type NF1-GRD (Figure 1).

This is consistent with the observation that NF201 and NF204 can effectively complement ira2$^-$ in yeast (see Table

1). On the other hand, the same extracts were not able to stimulate the GTPase activity of c-Ha-Ras^{Val12} under these experimental conditions. This suggests that the anti-oncogenic activity of the mutant NF1-GRD is not due to the stimulation of the slow GTPase of oncogenic Ras proteins.

The members of the GAP family negatively regulate the activity of Ras by stimulating intrinsic GTPase activity of normal Ras proteins. Thus, NF1 can potentially act as a specific block of effector function by normal Ras. However, oncogenic Ras lacks the intrinsic GTPase activity, and thus, natural GAP sequences cannot stimulate the inactivation of the activated oncogenic Ras. Likewise, NF1-GRD suppresses the heat shock-sensitive phenotype of *ira*$^-$ cells, but not the same phenotype of activated mutants of Ras, e.g., *RAS2*^{Val19} and *RAS2*^{Leu68} which correspond to mammalian oncogenic Ras, *ras*^{Val12} and *ras*^{Leu61}, respectively. Various mammalian oncogenic Ras mutants may be simulated by corresponding mutations in yeast Ras proteins. These observations lead to a model which is useful for testing interaction of Ras variants with GAP variants, and which predicts useful blocking or reversal of mutant or oncogenic Ras-induced effects.

A model of anti-oncogenic activity of mutant NF1-GRD consistent with these observations is that the mutant NF1-GRD has higher affinity for oncogenic Ras•GTP as compared to the wild-type NF1-GRD. As discussed above, the GAP binding region, and the effector binding regions on the Ras protein are in close physical proximity. As such, mutant NF1-GRD binding to oncogenic Ras, e.g., high affinity binding, could form an irreversible NF1•Ras•GTP complex. This could prevent interaction with putative downstream effector molecules, e.g., by conformational changes or competition. The oncogenic Ras would be sequestered from signal transduction pathways. Two observations support this hypothesis. First, as shown in Table 1, weak but significant phenotypic reversion of *RAS2*^{Leu68} by wild-type

NF1-GRD was observed. A previous study (Bollag et al. (1991) Nature 351:576-579) showed that the mammalian Ras^{Leu61} protein (corresponding to yeast $RAS2^{Leu68}$) has a much higher affinity for NF1-GRD than the wild-type or Val12-form of Ras. The high affinity binding between $RAS2^{Leu68}$ and wild-type NF1-GRD can explain the phenotypic suppression. Likewise, this model can also explain the differences in transformation-suppressor activities among GAP, wild-type NF1-GRD, and mutant NF1-GRDs. Table 2 shows that wild-type NF1-GRD, but not GAP, can suppress transformation by v-Ras; two mutant NF1-GRDs are more potent suppressors than wild-type NF1-GRD. This order of potency as transformation suppressors may reflect the relative affinity for Ras proteins; that is, wild-type NF1-GRD has 20 times higher affinity for Ras than GAP (see Martin et al. Cell 63:843-850); mutant NF1-GRDs may have even greater affinities. In relation to this, it should be noted that Ballester et al. (1990) Cell 63:851-859 previously observed the inhibitory effect of wild-type NF1-GRD but not of GAP on c-Ha-Ras^{Val12} expressed in yeast cells. This is consistent with the observation that wild-type NF1-GRD can weakly suppress v-Ras-transformation in mammalian cells. The second observation supporting this model is that NF201 can suppress the activity of not only $RAS2^{Val19}$ and $RAS2^{Leu68}$, but also $RAS2^{Ser41}$. It has been shown that Ser41 mutation (corresponding to Ser34 of human Ras), which is located in the so-called "effector region," disrupts the effective binding of Ras2 proteins to yeast Ira proteins as well as NF1-GRD and GAP (Tanaka et al. (1992) Mol. Cell. Biol. 12:631-637). Thus, the fact that NF201 can inhibit the activity of $RAS2^{Ser41}$ strongly suggests that the mutation in NF201 restores the interaction between $RAS2^{Ser41}$ and NF1-GRD. Comparison of the relative affinities of wild-type and mutant NF1-GRDs for oncogenic Ras proteins should provide a test for this model. This model predicts that highly specific reagents could be produced having specificity only for blocking

oncogenic Ras effects while having virtually no effects on normal Ras.

In summary, the data presented herein demonstrated that NF1-GRDs with single amino acid substitutions can suppress the biological activity of oncogenic Ras. According to the proposed model, mutant NF1-GRDs could inhibit specifically oncogenic but not normal Ras. In the case of normal Ras•GTP, bound GTP would be rapidly hydrolyzed to GDP upon interaction with NF1-GRD, and NF1-GRD would be released from Ras•GDP. In this study, a mutant NF1-GRD was expressed as a protein of 578 amino acids, which is still a substantially large protein. The yeast screening system described will allow determination of the minimum fragment of NF1-GRD which retains anti-oncogenic activity. This approach will allow development of Ras-specific anti-oncogenic compounds.

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

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(i) APPLICANT: Schering Corp.

(ii) TITLE OF INVENTION: RAS Associated GAP Proteins

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Schering Corp.
(B) STREET: 1 Girald Farms
(C) CITY: Madison
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) ZIP: 94304-1104

(v) COMPUTER READABLE FORM:
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(A) NAME: Lunn, Paul G.
(B) REGISTRATION NUMBER: 32,743
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(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (201)822-7255
(B) TELEFAX: (201)822-7039

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2485 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 564..9380

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Asn Trp Glu Asp Asn Ser Val Ile Phe Leu Leu Val Gln Ser Met Val
1               5                  10                  15

Val Asp Leu Lys Asn Leu Leu Phe Asn Pro Ser Lys Pro Phe Ser Arg
            20                  25                  30

Gly Ser Gln Pro Ala Asp Val Asp Leu Met Ile Asp Cys Leu Val Ser
        35                  40                  45

Cys Phe Arg Ile Ser Pro His Asn Asn Gln His Phe Lys Ile Cys Leu
    50                  55                  60

Ala Gln Asn Ser Pro Ser Thr Phe His Tyr Val Leu Val Asn Ser Leu
65                  70                  75                  80

His Arg Ile Ile Thr Asn Ser Ala Leu Asp Trp Trp Pro Lys Ile Asp
                85                  90                  95

Ala Val Tyr Cys His Ser Val Glu Leu Arg Asn Met Phe Gly Glu Thr
            100                 105                 110

Leu His Lys Ala Val Gln Gly Cys Gly Ala His Pro Ala Ile Arg Met
        115                 120                 125

Ala Pro Ser Leu Thr Phe Lys Glu Lys Val Thr Ser Leu Lys Phe Lys
    130                 135                 140

Glu Lys Pro Thr Asp Leu Glu Thr Arg Ser Tyr Lys Tyr Leu Leu Leu
145                 150                 155                 160

Ser Ile Val Lys Leu Ile His Ala Asp Pro Lys Leu Leu Leu Cys Asn
                165                 170                 175

Pro Arg Lys Gln Gly Pro Glu Thr Gln Gly Ser Thr Ala Glu Leu Ile
            180                 185                 190

Thr Gly Leu Val Gln Leu Val Pro Gln Ser His Met Pro Glu Ile Ala
        195                 200                 205

Gln Glu Ala Met Glu Ala Leu Leu Val Leu His Gln Leu Asp Ser Ile
    210                 215                 220

Asp Leu Trp Asn Pro Asp Ala Pro Val Glu Thr Phe Trp Glu Ile Ser
225                 230                 235                 240

Ser Gln Met Leu Phe Tyr Ile Cys Lys Lys Leu Thr Ser His Gln Met
                245                 250                 255

Leu Ser Ser Thr Glu Ile Leu Lys Trp Leu Arg Glu Ile Leu Ile Cys
            260                 265                 270

Arg Asn Lys Phe Leu Leu Lys Asn Lys Gln Ala Asp Arg Ser Ser Cys
        275                 280                 285

His Phe Leu Leu Phe Tyr Gly Val Gly Cys Asp Ile Pro Ser Ser Gly
    290                 295                 300

Asn Thr Ser Gln Met Ser Met Asp His Glu Glu Leu Leu Arg Thr Pro
305                 310                 315                 320
```

Gly Ala Ser Leu Arg Lys Gly Lys Gly Asn Ser Ser Met Asp Ser Ala
325 330 335

Ala Gly Cys Ser Gly Thr Pro Pro Ile Cys Arg Gln Ala Gln Thr Lys
340 345 350

Leu Glu Val Ala Leu Tyr Met Phe Leu Trp Asn Pro Asp Thr Glu Ala
355 360 365

Val Leu Val Ala Met Ser Cys Phe Arg His Leu Cys Glu Glu Ala Asp
370 375 380

Ile Arg Cys Gly Val Asp Glu Val Ser Val His Asn Leu Leu Pro Asn
385 390 395 400

Tyr Asn Thr Phe Met Glu Phe Ala Ser Val Ser Asn Met Met Ser Thr
405 410 415

Gly Arg Ala Ala Leu Gln Lys Arg Val Met Ala Leu Leu Arg Arg Ile
420 425 430

Glu His Pro Thr Ala Gly Asn Thr Glu Ala Trp Glu Asp Thr His Ala
435 440 445

Lys Trp Glu Gln Ala Thr Lys Leu Ile Leu Asn Tyr Pro Lys Ala Lys
450 455 460

Met Glu Asp Gly Gln Ala Ala Glu Ser Leu His Lys Thr Ile Val Lys
465 470 475 480

Arg Arg Met Ser His Val Ser Gly Gly Gly Ser Ile Asp Leu Ser Asp
485 490 495

Thr Asp Ser Leu Gln Glu Trp Ile Asn Met Thr Gly Phe Leu Cys Ala
500 505 510

Leu Gly Gly Val Cys Leu Gln Gln Arg Ser Asn Ser Gly Leu Ala Thr
515 520 525

Tyr Ser Pro Pro Met Gly Pro Val Ser Glu Arg Lys Gly Ser Met Ile
530 535 540

Ser Val Met Ser Ser Glu Gly Asn Ala Asp Thr Pro Val Ser Lys Phe
545 550 555 560

Met Asp Arg Leu Leu Ser Leu Met Val Cys Asn His Glu Lys Val Gly
565 570 575

Leu Gln Ile Arg Thr Asn Val Lys Asp Leu Val Gly Leu Glu Leu Ser
580 585 590

Pro Ala Leu Tyr Pro Met Leu Phe Asn Lys Leu Lys Asn Thr Ile Ser
595 600 605

Lys Phe Phe Asp Ser Gln Gly Gln Val Leu Leu Thr Asp Thr Asn Thr
610 615 620

Gln Phe Val Glu Gln Thr Ile Ala Ile Met Lys Asn Leu Leu Asp Asn
625 630 635 640

His Thr Glu Gly Ser Ser Glu His Leu Gly Gln Ala Ser Ile Glu Thr
645 650 655

Met Met Leu Asn Leu Val Arg Tyr Val Arg Val Leu Gly Asn Met Val
660 665 670

His Ala Ile Gln Ile Lys Thr Lys Leu Cys Gln Leu Val Glu Val Met
675 680 685

Met Ala Arg Arg Asp Asp Leu Ser Phe Cys Gln Glu Met Lys Phe Arg
690 695 700

Asn Lys Met Val Glu Tyr Leu Thr Asp Trp Val Met Gly Thr Ser Asn
705 710 715 720

Gln Ala Ala Asp Asp Asp Val Lys Cys Leu Thr Arg Asp Leu Asp Gln
725 730 735

Ala Ser Met Glu Ala Val Val Ser Leu Leu Ala Gly Leu Pro Leu Gln
740 745 750

Pro Glu Glu Gly Asp Gly Val Glu Leu Met Glu Ala Lys Ser Gln Leu
755 760 765

Phe Leu Lys Tyr Phe Thr Leu Phe Met Asn Leu Leu Asn Asp Cys Ser
770 775 780

Glu Val Glu Asp Glu Ser Ala Gln Thr Gly Gly Arg Lys Arg Gly Met
785 790 795 800

Ser Arg Arg Leu Ala Ser Leu Arg His Cys Thr Val Leu Ala Met Ser
805 810 815

Asn Leu Leu Asn Ala Asn Val Asp Ser Gly Leu Met His Ser Ile Gly
820 825 830

Leu Gly Tyr His Lys Asp Leu Gln Thr Arg Ala Thr Phe Met Glu Val
835 840 845

Leu Thr Lys Ile Leu Gln Gln Gly Thr Glu Phe Asp Thr Leu Ala Glu
850 855 860

Thr Val Leu Ala Asp Arg Phe Glu Arg Leu Val Glu Leu Val Thr Met
865 870 875 880

Met Gly Asp Gln Gly Glu Leu Pro Ile Ala Met Ala Leu Ala Asn Val
885 890 895

Val Pro Cys Ser Gln Trp Asp Glu Leu Ala Arg Val Leu Val Thr Leu
900 905 910

Phe Asp Ser Arg His Leu Leu Tyr Gln Leu Leu Trp Asn Met Phe Ser
915 920 925

Lys Glu Val Glu Leu Ala Asp Ser Met Gln Thr Leu Phe Arg Gly Asn
930 935 940

Ser Leu Ala Ser Lys Ile Met Thr Phe Cys Phe Lys Val Tyr Gly Ala
945 950 955 960

Thr Tyr Leu Gln Lys Leu Leu Asp Pro Leu Leu Arg Ile Val Ile Thr
965 970 975

Ser Ser Asp Trp Gln His Val Ser Phe Glu Val Asp Pro Thr Arg Leu
980 985 990

Glu Pro Ser Glu Ser Leu Glu Glu Asn Gln Arg Asn Leu Leu Gln Met
995 1000 1005

Thr Glu Lys Phe Phe His Ala Ile Ile Ser Ser Ser Ser Glu Phe Pro
1010 1015 1020

Pro Gln Leu Arg Ser Val Cys His Cys Leu Tyr Gln Val Val Ser Gln
1025 1030 1035 1040

Arg Phe Pro Gln Asn Ser Ile Gly Ala Val Gly Ser Ala Met Phe Leu
1045 1050 1055

Arg Phe Ile Asn Pro Ala Ile Val Ser Pro Tyr Glu Ala Gly Ile Leu
1060 1065 1070

Asp Lys Lys Pro Pro Pro Arg Ile Glu Arg Gly Leu Lys Leu Met Ser
1075 1080 1085

Lys Ile Leu Gln Ser Ile Ala Asn His Val Leu Leu Thr Lys Glu Glu
1090 1095 1100

His Met Arg Pro Phe Asn Asp Phe Val Lys Ser Asn Phe Asp Ala Ala
1105 1110 1115 1120

Arg Arg Phe Phe Leu Asp Ile Ala Ser Asp Cys Pro Thr Ser Asp Ala
1125 1130 1135

Val Asn His Ser Leu Ser Phe Ile Ser Asp Gly Asn Val Leu Ala Leu
1140 1145 1150

His Arg Leu Leu Trp Asn Asn Gln Glu Lys Ile Gly Gln Tyr Leu Ser
1155 1160 1165

Ser Asn Arg Asp His Lys Ala Val Gly Arg Arg Pro Phe Asp Lys Met
1170 1175 1180

Ala Thr Leu Leu Ala Tyr Leu Gly Pro Pro Glu His Lys Pro Val Ala
1185 1190 1195 1200

Asp Thr His Trp Ser Ser Leu Asn Leu Thr Ser Ser Lys Phe Glu Glu
1205 1210 1215

Phe Met Thr Arg His His Gln Val His Glu Lys Glu Glu Phe Lys Ala
1220 1225 1230

Leu Lys Thr Leu Ser Ile Phe Tyr Gln Ala Gly Thr Ser Lys Ala Gly
1235 1240 1245

Asn Pro Ile Phe Tyr Tyr Val Ala Arg Arg Phe Lys Thr Gly Gln Ile
1250 1255 1260

Asn Gly Asp Leu Leu Ile Tyr His Val Leu Leu Thr Leu Lys Pro Tyr
1265 1270 1275 1280

Tyr Ala Lys Pro Tyr Glu Ile Val Val Asp Leu Thr His Thr Gly Pro
1285 1290 1295

Ser Asn Arg Phe Lys Thr Asp Phe Leu Ser Lys Trp Phe Val Val Phe
1300 1305 1310

Pro Gly Phe Ala Tyr Asp Asn Val Ser Ala Val Tyr Ile Tyr Asn Cys
1315 1320 1325

Asn Ser Trp Val Arg Glu Tyr Thr Lys Tyr His Glu Arg Leu Leu Thr
1330 1335 1340

Gly Leu Lys Gly Ser Lys Arg Leu Val Phe Ile Asp Cys Pro Gly Lys
1345 1350 1355 1360

Leu Ala Glu His Ile Glu His Glu Gln Gln Lys Leu Pro Ala Ala Thr
1365 1370 1375

Leu Ala Leu Glu Glu Asp Leu Lys Val Phe His Asn Ala Leu Lys Leu
1380 1385 1390

Ala His Lys Asp Thr Lys Val Ser Ile Lys Val Gly Ser Thr Ala Val
1395 1400 1405

Gln Val Thr Ser Ala Glu Arg Thr Lys Val Leu Gly Gln Ser Val Phe
1410 1415 1420

Leu Asn Asp Ile Tyr Tyr Ala Ser Glu Ile Glu Glu Ile Cys Leu Val
1425 1430 1435 1440

Asp Glu Asn Gln Phe Thr Leu Thr Ile Ala Asn Gln Gly Thr Pro Leu
1445 1450 1455

Thr Phe Met His Gln Glu Cys Glu Ala Ile Val Gln Ser Ile Ile His
1460 1465 1470

Ile Arg Thr Arg Trp Glu Leu Ser Gln Pro Asp Ser Ile Pro Gln His
1475 1480 1485

Thr Lys Ile Arg Pro Lys Asp Val Pro Gly Thr Leu Leu Asn Ile Ala
1490 1495 1500

Leu Leu Asn Leu Gly Ser Ser Asp Pro Ser Leu Arg Ser Ala Ala Tyr
1505 1510 1515 1520

Asn Leu Leu Cys Ala Leu Thr Cys Thr Phe Asn Leu Lys Ile Glu Gly
1525 1530 1535

Gln Leu Leu Glu Thr Ser Gly Leu Cys Ile Pro Ala Asn Asn Thr Leu
1540 1545 1550

Phe Ile Val Ser Ile Ser Lys Thr Leu Ala Ala Asn Glu Pro His Leu
1555 1560 1565

Thr Leu Glu Phe Leu Glu Glu Cys Ile Ser Gly Phe Ser Lys Ser Ser
1570 1575 1580

Ile Glu Leu Lys His Leu Cys Leu Glu Tyr Met Thr Pro Trp Leu Ser
1585 1590 1595 1600

Asn Leu Val Arg Phe Cys Lys His Asn Asp Asp Ala Lys Arg Gln Arg
1605 1610 1615

Val Thr Ala Ile Leu Asp Lys Leu Ile Thr Met Thr Ile Asn Glu Lys
1620 1625 1630

Gln Met Tyr Pro Ser Ile Gln Ala Lys Ile Trp Gly Ser Leu Gly Gln
1635 1640 1645

Ile Thr Asp Leu Leu Asp Val Val Leu Asp Ser Phe Ile Lys Thr Ser
1650 1655 1660

Ala Thr Gly Gly Leu Gly Ser Ile Lys Ala Glu Val Met Ala Asp Thr
1665 1670 1675 1680

Ala Val Ala Leu Ala Ser Gly Asn Val Lys Leu Val Ser Ser Lys Val
1685 1690 1695

Ile Gly Arg Met Cys Lys Ile Ile Asp Lys Thr Cys Leu Ser Pro Thr
1700 1705 1710

Pro Thr Leu Glu Gln His Leu Met Trp Asp Asp Ile Ala Ile Leu Ala
1715 1720 1725

Arg Tyr Met Leu Met Leu Ser Phe Asn Asn Ser Leu Asp Val Ala Ala
1730 1735 1740

His Leu Pro Tyr Leu Phe His Val Val Thr Phe Leu Val Ala Thr Gly
1745 1750 1755 1760

Pro Leu Ser Leu Arg Ala Ser Thr His Gly Leu Val Ile Asn Ile Ile
1765 1770 1775

His Ser Leu Cys Thr Cys Ser Gln Leu His Phe Ser Glu Glu Thr Lys
1780 1785 1790

Gln Val Leu Arg Leu Ser Leu Thr Glu Phe Ser Leu Pro Lys Phe Tyr
1795 1800 1805

Leu Leu Phe Gly Ile Ser Lys Val Lys Ser Ala Ala Val Ile Ala Phe
1810 1815 1820

Arg Ser Ser Tyr Arg Asp Arg Ser Phe Ser Pro Gly Ser Tyr Glu Arg
1825 1830 1835 1840

Glu Thr Phe Ala Leu Thr Ser Leu Glu Thr Val Thr Glu Ala Leu Leu
1845 1850 1855

Glu Ile Met Glu Ala Cys Met Arg Asp Ile Pro Thr Cys Lys Trp Leu
1860 1865 1870

Asp Gln Trp Thr Glu Leu Ala Gln Arg Phe Ala Phe Gln Tyr Asn Pro
1875 1880 1885

Ser Leu Gln Pro Arg Ala Leu Val Val Phe Gly Cys Ile Ser Lys Arg
1890 1895 1900

Val Ser His Gly Gln Ile Lys Gln Ile Ile Arg Ile Leu Ser Lys Ala
1905 1910 1915 1920

Leu Glu Ser Cys Leu Lys Gly Pro Asp Thr Tyr Asn Ser Gln Val Leu
1925 1930 1935

Ile Glu Ala Thr Val Ile Ala Leu Thr Lys Leu Gln Pro Leu Leu Asn
1940 1945 1950

Lys Asp Ser Pro Leu His Lys Ala Leu Phe Trp Val Ala Val Ala Val
1955 1960 1965

Leu Gln Leu Asp Glu Val Asn Leu Tyr Ser Ala Gly Thr Ala Leu Leu
1970 1975 1980

Glu Gln Asn Leu His Thr Leu Asp Ser Leu Arg Ile Phe Asn Asp Lys
1985 1990 1995 2000

Ser Pro Glu Glu Val Phe Met Ala Ile Arg Asn Pro Leu Glu Trp His
2005 2010 2015

Cys Lys Gln Met Asp His Phe Val Gly Leu Asn Phe Asn Ser Asn Phe
2020 2025 2030

Asn Phe Ala Leu Val Gly His Leu Leu Lys Gly Tyr Arg His Pro Ser
2035 2040 2045

Pro Ala Ile Val Ala Arg Thr Val Arg Ile Leu His Thr Leu Leu Thr
2050 2055 2060

Leu Val Asn Lys His Arg Asn Cys Asp Lys Phe Glu Val Asn Thr Gln
2065 2070 2075 2080

Ser Val Ala Tyr Leu Ala Ala Leu Leu Thr Val Ser Glu Glu Val Arg
2085 2090 2095

Ser Arg Cys Ser Leu Lys His Arg Lys Ser Leu Leu Leu Thr Asp Ile
2100 2105 2110

Ser Met Glu Asn Val Pro Met Asp Thr Tyr Pro Ile His His Gly Asp
2115 2120 2125

Pro Ser Tyr Arg Thr Leu Lys Glu Thr Gln Pro Trp Ser Ser Pro Lys
2130 2135 2140

Gly Ser Glu Gly Tyr Leu Ala Ala Thr Tyr Pro Thr Val Gly Gln Thr
2145 2150 2155 2160

Ser Pro Arg Ala Arg Lys Ser Met Ser Leu Asp Met Gly Gln Pro Ser
2165 2170 2175

Gln Ala Asn Thr Lys Lys Leu Leu Gly Thr Arg Lys Ser Phe Asp His
2180 2185 2190

Leu Ile Ser Asp Thr Lys Ala Pro Lys Arg Gln Glu Met Glu Ser Gly
2195 2200 2205

Ile Thr Thr Pro Pro Lys Met Arg Arg Val Ala Glu Thr Asp Tyr Glu
2210 2215 2220

Met Glu Thr Gln Arg Ile Ser Ser Ser Gln Gln His Pro His Leu Arg
2225 2230 2235 2240

Lys Val Ser Val Ser Glu Ser Asn Val Leu Leu Asp Glu Glu Val Leu
2245 2250 2255

Thr Asp Pro Lys Ile Gln Ala Leu Leu Leu Thr Val Leu Ala Thr Leu
2260 2265 2270

Val Lys Tyr Thr Thr Asp Glu Phe Asp Gln Arg Ile Leu Tyr Glu Tyr
2275 2280 2285

Leu Ala Glu Ala Ser Val Val Phe Pro Lys Val Phe Pro Val Val His
2290 2295 2300

Asn Leu Leu Asp Ser Lys Ile Asn Thr Leu Leu Ser Leu Cys Gln Asp
2305 2310 2315 2320

Pro Asn Leu Leu Asn Pro Ile His Gly Ile Val Gln Ser Val Val Tyr
2325 2330 2335

His Glu Glu Ser Pro Pro Gln Tyr Gln Thr Ser Tyr Leu Gln Ser Phe
2340 2345 2350

Gly Phe Asn Gly Leu Trp Arg Phe Ala Gly Pro Phe Ser Lys Gln Thr
2355 2360 2365

Gln Ile Pro Asp Tyr Ala Glu Leu Ile Val Lys Phe Leu Asp Ala Leu
2370 2375 2380

Ile Asp Thr Tyr Leu Pro Gly Ile Asp Glu Glu Thr Ser Glu Glu Ser
2385 2390 2395 2400

Leu Leu Thr Pro Thr Ser Pro Tyr Pro Pro Ala Leu Gln Ser Gln Leu
2405 2410 2415

Ser Ile Thr Ala Asn Leu Asn Leu Ser Asn Ser Met Thr Ser Leu Ala
2420 2425 2430

Thr Ser Gln His Ser Pro Gly Ile Asp Lys Glu Asn Val Glu Leu Ser
2435 2440 2445

Pro Thr Thr Gly His Cys Asn Ser Gly Arg Thr Arg His Gly Ser Ala
2450 2455 2460

Ser Gln Val Gln Lys Gln Arg Ser Ala Gly Ser Phe Lys Arg Asn Ser
2465 2470 2475 2480

Ile Lys Lys Ile Val
2485

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2485 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asn Trp Glu Asp Asn Ser Val Ile Phe Leu Leu Val Gln Ser Met Val
1 5 10 15

Val Asp Leu Lys Asn Leu Leu Phe Asn Pro Ser Lys Pro Phe Ser Arg
20 25 30

Gly Ser Gln Pro Ala Asp Val Asp Leu Met Ile Asp Cys Leu Val Ser
35 40 45

Cys Phe Arg Ile Ser Pro His Asn Asn Gln His Phe Lys Ile Cys Leu
50 55 60

Ala Gln Asn Ser Pro Ser Thr Phe His Tyr Val Leu Val Asn Ser Leu
65 70 75 80

His Arg Ile Ile Thr Asn Ser Ala Leu Asp Trp Trp Pro Lys Ile Asp
85 90 95

Ala Val Tyr Cys His Ser Val Glu Leu Arg Asn Met Phe Gly Glu Thr
100 105 110

Leu His Lys Ala Val Gln Gly Cys Gly Ala His Pro Ala Ile Arg Met
115 120 125

Ala Pro Ser Leu Thr Phe Lys Glu Lys Val Thr Ser Leu Lys Phe Lys
130 135 140

Glu Lys Pro Thr Asp Leu Glu Thr Arg Ser Tyr Lys Tyr Leu Leu Leu
145 150 155 160

Ser Ile Val Lys Leu Ile His Ala Asp Pro Lys Leu Leu Leu Cys Asn
165 170 175

Pro Arg Lys Gln Gly Pro Glu Thr Gln Gly Ser Thr Ala Glu Leu Ile
180 185 190

Thr Gly Leu Val Gln Leu Val Pro Gln Ser His Met Pro Glu Ile Ala
195 200 205

Gln Glu Ala Met Glu Ala Leu Leu Val Leu His Gln Leu Asp Ser Ile
210 215 220

Asp Leu Trp Asn Pro Asp Ala Pro Val Glu Thr Phe Trp Glu Ile Ser
225 230 235 240

Ser Gln Met Leu Phe Tyr Ile Cys Lys Lys Leu Thr Ser His Gln Met
245 250 255

Leu Ser Ser Thr Glu Ile Leu Lys Trp Leu Arg Glu Ile Leu Ile Cys
260 265 270

Arg Asn Lys Phe Leu Leu Lys Asn Lys Gln Ala Asp Arg Ser Ser Cys
275 280 285

His Phe Leu Leu Phe Tyr Gly Val Gly Cys Asp Ile Pro Ser Ser Gly
290 295 300

Asn Thr Ser Gln Met Ser Met Asp His Glu Glu Leu Leu Arg Thr Pro
305 310 315 320

Gly Ala Ser Leu Arg Lys Gly Lys Gly Asn Ser Ser Met Asp Ser Ala
325 330 335

Ala Gly Cys Ser Gly Thr Pro Pro Ile Cys Arg Gln Ala Gln Thr Lys
340 345 350

Leu Glu Val Ala Leu Tyr Met Phe Leu Trp Asn Pro Asp Thr Glu Ala
355 360 365

Val Leu Val Ala Met Ser Cys Phe Arg His Leu Cys Glu Glu Ala Asp
370 375 380

Ile Arg Cys Gly Val Asp Glu Val Ser Val His Asn Leu Leu Pro Asn
385 390 395 400

Tyr Asn Thr Phe Met Glu Phe Ala Ser Val Ser Asn Met Met Ser Thr
405 410 415

Gly Arg Ala Ala Leu Gln Lys Arg Val Met Ala Leu Leu Arg Arg Ile
420 425 430

Glu His Pro Thr Ala Gly Asn Thr Glu Ala Trp Glu Asp Thr His Ala
435 440 445

Lys Trp Glu Gln Ala Thr Lys Leu Ile Leu Asn Tyr Pro Lys Ala Lys
450 455 460

Met Glu Asp Gly Gln Ala Ala Glu Ser Leu His Lys Thr Ile Val Lys
465 470 475 480

Arg Arg Met Ser His Val Ser Gly Gly Gly Ser Ile Asp Leu Ser Asp
485 490 495

Thr Asp Ser Leu Gln Glu Trp Ile Asn Met Thr Gly Phe Leu Cys Ala
500 505 510

Leu Gly Gly Val Cys Leu Gln Gln Arg Ser Asn Ser Gly Leu Ala Thr
515 520 525

Tyr Ser Pro Pro Met Gly Pro Val Ser Glu Arg Lys Gly Ser Met Ile
530 535 540

Ser Val Met Ser Ser Glu Gly Asn Ala Asp Thr Pro Val Ser Lys Phe
545 550 555 560

Met Asp Arg Leu Leu Ser Leu Met Val Cys Asn His Glu Lys Val Gly
565 570 575

Leu Gln Ile Arg Thr Asn Val Lys Asp Leu Val Gly Leu Glu Leu Ser
580 585 590

Pro Ala Leu Tyr Pro Met Leu Phe Asn Lys Leu Lys Asn Thr Ile Ser
595 600 605

Lys Phe Phe Asp Ser Gln Gly Gln Val Leu Leu Thr Asp Thr Asn Thr
610 615 620

Gln Phe Val Glu Gln Thr Ile Ala Ile Met Lys Asn Leu Leu Asp Asn
625 630 635 640

His Thr Glu Gly Ser Ser Glu His Leu Gly Gln Ala Ser Ile Glu Thr
645 650 655

Met Met Leu Asn Leu Val Arg Tyr Val Arg Val Leu Gly Asn Met Val
660 665 670

His Ala Ile Gln Ile Lys Thr Lys Leu Cys Gln Leu Val Glu Val Met
675 680 685

Met Ala Arg Arg Asp Asp Leu Ser Phe Cys Gln Glu Met Lys Phe Arg
690 695 700

Asn Lys Met Val Glu Tyr Leu Thr Asp Trp Val Met Gly Thr Ser Asn
705 710 715 720

Gln Ala Ala Asp Asp Asp Val Lys Cys Leu Thr Arg Asp Leu Asp Gln
725 730 735

Ala Ser Met Glu Ala Val Val Ser Leu Leu Ala Gly Leu Pro Leu Gln
740 745 750

Pro Glu Glu Gly Asp Gly Val Glu Leu Met Glu Ala Lys Ser Gln Leu
755 760 765

Phe Leu Lys Tyr Phe Thr Leu Phe Met Asn Leu Leu Asn Asp Cys Ser
770 775 780

Glu Val Glu Asp Glu Ser Ala Gln Thr Gly Gly Arg Lys Arg Gly Met
785 790 795 800

Ser Arg Arg Leu Ala Ser Leu Arg His Cys Thr Val Leu Ala Met Ser
805 810 815

Asn Leu Leu Asn Ala Asn Val Asp Ser Gly Leu Met His Ser Ile Gly
820 825 830

Leu Gly Tyr His Lys Asp Leu Gln Thr Arg Ala Thr Phe Met Glu Val
835 840 845

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Leu Thr Lys Ile Leu Gln Gln Gly Thr Glu Phe Asp Thr Leu Ala Glu
     850                 855                 860

Thr Val Leu Ala Asp Arg Phe Glu Arg Leu Val Glu Leu Val Thr Met
865                 870                 875                 880

Met Gly Asp Gln Gly Glu Leu Pro Ile Ala Met Ala Leu Ala Asn Val
                885                 890                 895

Val Pro Cys Ser Gln Trp Asp Glu Leu Ala Arg Val Leu Val Thr Leu
            900                 905                 910

Phe Asp Ser Arg His Leu Leu Tyr Gln Leu Leu Trp Asn Met Phe Ser
        915                 920                 925

Lys Glu Val Glu Leu Ala Asp Ser Met Gln Thr Leu Phe Arg Gly Asn
    930                 935                 940

Ser Leu Ala Ser Lys Ile Met Thr Phe Cys Phe Lys Val Tyr Gly Ala
945                 950                 955                 960

Thr Tyr Leu Gln Lys Leu Leu Asp Pro Leu Leu Arg Ile Val Ile Thr
                965                 970                 975

Ser Ser Asp Trp Gln His Val Ser Phe Glu Val Asp Pro Thr Arg Leu
            980                 985                 990

Glu Pro Ser Glu Ser Leu Glu Glu Asn Gln Arg Asn Leu Leu Gln Met
        995                 1000                1005

Thr Glu Lys Phe Phe His Ala Ile Ile Ser Ser Ser Ser Glu Phe Pro
    1010                1015                1020

Pro Gln Leu Arg Ser Val Cys His Cys Leu Tyr Gln Val Val Ser Gln
1025                1030                1035                1040

Arg Phe Pro Gln Asn Ser Ile Gly Ala Val Gly Ser Ala Met Phe Leu
                1045                1050                1055

Arg Phe Ile Asn Pro Ala Ile Val Ser Pro Tyr Glu Ala Gly Ile Leu
            1060                1065                1070

Asp Lys Lys Pro Pro Pro Arg Ile Glu Arg Gly Leu Lys Leu Met Ser
        1075                1080                1085

Lys Ile Leu Gln Ser Ile Ala Asn His Val Leu Phe Thr Arg Glu Glu
    1090                1095                1100

His Met Arg Pro Phe Asn Asp Phe Val Lys Ser Asn Phe Asp Ala Ala
1105                1110                1115                1120

Arg Arg Phe Phe Leu Asp Ile Ala Ser Asp Cys Pro Thr Ser Asp Ala
                1125                1130                1135

Val Asn His Ser Leu Ser Phe Ile Ser Asp Gly Asn Val Leu Ala Leu
            1140                1145                1150

His Arg Leu Leu Trp Asn Asn Gln Glu Lys Ile Gly Gln Tyr Leu Ser
        1155                1160                1165
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Ser Asn Arg Asp His Lys Ala Val Gly Arg Arg Pro Phe Asp Lys Met
1170 1175 1180

Ala Thr Leu Leu Ala Tyr Leu Gly Pro Pro Glu His Lys Pro Val Ala
1185 1190 1195 1200

Asp Thr His Trp Ser Ser Leu Asn Leu Thr Ser Ser Lys Phe Glu Glu
1205 1210 1215

Phe Met Thr Arg His His Gln Val His Glu Lys Glu Glu Phe Lys Ala
1220 1225 1230

Leu Lys Thr Leu Ser Ile Phe Tyr Gln Ala Gly Thr Ser Lys Ala Gly
1235 1240 1245

Asn Pro Ile Phe Tyr Tyr Val Ala Arg Arg Phe Lys Thr Gly Gln Ile
1250 1255 1260

Asn Gly Asp Leu Leu Ile Tyr His Val Leu Leu Thr Leu Lys Pro Tyr
1265 1270 1275 1280

Tyr Ala Lys Pro Tyr Glu Ile Val Val Asp Leu Thr His Thr Gly Pro
1285 1290 1295

Ser Asn Arg Phe Lys Thr Asp Phe Leu Ser Lys Trp Phe Val Val Phe
1300 1305 1310

Pro Gly Phe Ala Tyr Asp Asn Val Ser Ala Val Tyr Ile Tyr Asn Cys
1315 1320 1325

Asn Ser Trp Val Arg Glu Tyr Thr Lys Tyr His Glu Arg Leu Leu Thr
1330 1335 1340

Gly Leu Lys Gly Ser Lys Arg Leu Val Phe Ile Asp Cys Pro Gly Lys
1345 1350 1355 1360

Leu Ala Glu His Ile Glu His Glu Gln Gln Lys Leu Pro Ala Ala Thr
1365 1370 1375

Leu Ala Leu Glu Glu Asp Leu Lys Val Phe His Asn Ala Leu Lys Leu
1380 1385 1390

Ala His Lys Asp Thr Lys Val Ser Ile Lys Val Gly Ser Thr Ala Val
1395 1400 1405

Gln Val Thr Ser Ala Glu Arg Thr Lys Val Leu Gly Gln Ser Val Phe
1410 1415 1420

Leu Asn Asp Ile Tyr Tyr Ala Ser Glu Ile Glu Glu Ile Cys Leu Val
1425 1430 1435 1440

Asp Glu Asn Gln Phe Thr Leu Thr Ile Ala Asn Gln Gly Thr Pro Leu
1445 1450 1455

Thr Phe Met His Gln Glu Cys Glu Ala Ile Val Gln Ser Ile Ile His
1460 1465 1470

Ile Arg Thr Arg Trp Glu Leu Ser Gln Pro Asp Ser Ile Pro Gln His
1475 1480 1485

Thr Lys Ile Arg Pro Lys Asp Val Pro Gly Thr Leu Leu Asn Ile Ala
1490 1495 1500

Leu Leu Asn Leu Gly Ser Ser Asp Pro Ser Leu Arg Ser Ala Ala Tyr
1505 1510 1515 1520

Asn Leu Leu Cys Ala Leu Thr Cys Thr Phe Asn Leu Lys Ile Glu Gly
1525 1530 1535

Gln Leu Leu Glu Thr Ser Gly Leu Cys Ile Pro Ala Asn Asn Thr Leu
1540 1545 1550

Phe Ile Val Ser Ile Ser Lys Thr Leu Ala Ala Asn Glu Pro His Leu
1555 1560 1565

Thr Leu Glu Phe Leu Glu Glu Cys Ile Ser Gly Phe Ser Lys Ser Ser
1570 1575 1580

Ile Glu Leu Lys His Leu Cys Leu Glu Tyr Met Thr Pro Trp Leu Ser
1585 1590 1595 1600

Asn Leu Val Arg Phe Cys Lys His Asn Asp Asp Ala Lys Arg Gln Arg
1605 1610 1615

Val Thr Ala Ile Leu Asp Lys Leu Ile Thr Met Thr Ile Asn Glu Lys
1620 1625 1630

Gln Met Tyr Pro Ser Ile Gln Ala Lys Ile Trp Gly Ser Leu Gly Gln
1635 1640 1645

Ile Thr Asp Leu Leu Asp Val Val Leu Asp Ser Phe Ile Lys Thr Ser
1650 1655 1660

Ala Thr Gly Gly Leu Gly Ser Ile Lys Ala Glu Val Met Ala Asp Thr
1665 1670 1675 1680

Ala Val Ala Leu Ala Ser Gly Asn Val Lys Leu Val Ser Ser Lys Val
1685 1690 1695

Ile Gly Arg Met Cys Lys Ile Ile Asp Lys Thr Cys Leu Ser Pro Thr
1700 1705 1710

Pro Thr Leu Glu Gln His Leu Met Trp Asp Asp Ile Ala Ile Leu Ala
1715 1720 1725

Arg Tyr Met Leu Met Leu Ser Phe Asn Asn Ser Leu Asp Val Ala Ala
1730 1735 1740

His Leu Pro Tyr Leu Phe His Val Val Thr Phe Leu Val Ala Thr Gly
1745 1750 1755 1760

Pro Leu Ser Leu Arg Ala Ser Thr His Gly Leu Val Ile Asn Ile Ile
1765 1770 1775

His Ser Leu Cys Thr Cys Ser Gln Leu His Phe Ser Glu Glu Thr Lys
1780 1785 1790

Gln Val Leu Arg Leu Ser Leu Thr Glu Phe Ser Leu Pro Lys Phe Tyr
1795 1800 1805

Leu Leu Phe Gly Ile Ser Lys Val Lys Ser Ala Ala Val Ile Ala Phe
1810 1815 1820

Arg Ser Ser Tyr Arg Asp Arg Ser Phe Ser Pro Gly Ser Tyr Glu Arg
1825 1830 1835 1840

Glu Thr Phe Ala Leu Thr Ser Leu Glu Thr Val Thr Glu Ala Leu Leu
1845 1850 1855

Glu Ile Met Glu Ala Cys Met Arg Asp Ile Pro Thr Cys Lys Trp Leu
1860 1865 1870

Asp Gln Trp Thr Glu Leu Ala Gln Arg Phe Ala Phe Gln Tyr Asn Pro
1875 1880 1885

Ser Leu Gln Pro Arg Ala Leu Val Val Phe Gly Cys Ile Ser Lys Arg
1890 1895 1900

Val Ser His Gly Gln Ile Lys Gln Ile Ile Arg Ile Leu Ser Lys Ala
1905 1910 1915 1920

Leu Glu Ser Cys Leu Lys Gly Pro Asp Thr Tyr Asn Ser Gln Val Leu
1925 1930 1935

Ile Glu Ala Thr Val Ile Ala Leu Thr Lys Leu Gln Pro Leu Leu Asn
1940 1945 1950

Lys Asp Ser Pro Leu His Lys Ala Leu Phe Trp Val Ala Val Ala Val
1955 1960 1965

Leu Gln Leu Asp Glu Val Asn Leu Tyr Ser Ala Gly Thr Ala Leu Leu
1970 1975 1980

Glu Gln Asn Leu His Thr Leu Asp Ser Leu Arg Ile Phe Asn Asp Lys
1985 1990 1995 2000

Ser Pro Glu Glu Val Phe Met Ala Ile Arg Asn Pro Leu Glu Trp His
2005 2010 2015

Cys Lys Gln Met Asp His Phe Val Gly Leu Asn Phe Asn Ser Asn Phe
2020 2025 2030

Asn Phe Ala Leu Val Gly His Leu Leu Lys Gly Tyr Arg His Pro Ser
2035 2040 2045

Pro Ala Ile Val Ala Arg Thr Val Arg Ile Leu His Thr Leu Leu Thr
2050 2055 2060

Leu Val Asn Lys His Arg Asn Cys Asp Lys Phe Glu Val Asn Thr Gln
2065 2070 2075 2080

Ser Val Ala Tyr Leu Ala Ala Leu Leu Thr Val Ser Glu Glu Val Arg
2085 2090 2095

Ser Arg Cys Ser Leu Lys His Arg Lys Ser Leu Leu Leu Thr Asp Ile
2100 2105 2110

Ser Met Glu Asn Val Pro Met Asp Thr Tyr Pro Ile His His Gly Asp
2115 2120 2125

Pro Ser Tyr Arg Thr Leu Lys Glu Thr Gln Pro Trp Ser Ser Pro Lys
2130 2135 2140

Gly Ser Glu Gly Tyr Leu Ala Ala Thr Tyr Pro Thr Val Gly Gln Thr
2145 2150 2155 2160

Ser Pro Arg Ala Arg Lys Ser Met Ser Leu Asp Met Gly Gln Pro Ser
2165 2170 2175

Gln Ala Asn Thr Lys Lys Leu Leu Gly Thr Arg Lys Ser Phe Asp His
2180 2185 2190

Leu Ile Ser Asp Thr Lys Ala Pro Lys Arg Gln Glu Met Glu Ser Gly
2195 2200 2205

Ile Thr Thr Pro Pro Lys Met Arg Arg Val Ala Glu Thr Asp Tyr Glu
2210 2215 2220

Met Glu Thr Gln Arg Ile Ser Ser Ser Gln Gln His Pro His Leu Arg
2225 2230 2235 2240

Lys Val Ser Val Ser Glu Ser Asn Val Leu Leu Asp Glu Glu Val Leu
2245 2250 2255

Thr Asp Pro Lys Ile Gln Ala Leu Leu Leu Thr Val Leu Ala Thr Leu
2260 2265 2270

Val Lys Tyr Thr Thr Asp Glu Phe Asp Gln Arg Ile Leu Tyr Glu Tyr
2275 2280 2285

Leu Ala Glu Ala Ser Val Val Phe Pro Lys Val Phe Pro Val Val His
2290 2295 2300

Asn Leu Leu Asp Ser Lys Ile Asn Thr Leu Leu Ser Leu Cys Gln Asp
2305 2310 2315 2320

Pro Asn Leu Leu Asn Pro Ile His Gly Ile Val Gln Ser Val Val Tyr
2325 2330 2335

His Glu Glu Ser Pro Pro Gln Tyr Gln Thr Ser Tyr Leu Gln Ser Phe
2340 2345 2350

Gly Phe Asn Gly Leu Trp Arg Phe Ala Gly Pro Phe Ser Lys Gln Thr
2355 2360 2365

Gln Ile Pro Asp Tyr Ala Glu Leu Ile Val Lys Phe Leu Asp Ala Leu
2370 2375 2380

Ile Asp Thr Tyr Leu Pro Gly Ile Asp Glu Glu Thr Ser Glu Glu Ser
2385 2390 2395 2400

Leu Leu Thr Pro Thr Ser Pro Tyr Pro Pro Ala Leu Gln Ser Gln Leu
2405 2410 2415

Ser Ile Thr Ala Asn Leu Asn Leu Ser Asn Ser Met Thr Ser Leu Ala
2420 2425 2430

Thr Ser Gln His Ser Pro Ala Ser Leu Pro Cys Ser Asn Ser Ala Val
2435 2440 2445

Phe Met Gln Leu Phe Pro His Gln Gly Ile Asp Lys Glu Asn Val Glu
2450 2455 2460

Leu Ser Pro Thr Thr Gly His Cys Asn Ser Gly Arg Thr Arg His Gly
2465 2470 2475 2480

Ser Ala Ser Gln Val
5 2485

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2938 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Leu Leu Cys Lys Ile Ser Lys Leu Lys Phe Asn Thr Arg Thr Leu
1               5                  10                  15

Lys Val Leu Gln Asn Met Ser His His Leu Ser Gly Ser Ala Thr Ile
            20                  25                  30

Ser Lys Ser Ser Ile Leu Pro Asp Ser Gln Glu Phe Leu Gln Lys Arg
        35                  40                  45

Asn Tyr Pro Ala Tyr Thr Glu Lys Ile Asp Leu Thr Ile Asp Tyr Ile
    50                  55                  60

Gln Arg Phe Ile Ser Ala Ser Asn His Val Glu Phe Thr Lys Cys Val
65                  70                  75                  80

Lys Thr Lys Val Val Ala Pro Leu Leu Ile Ser His Thr Ser Thr Glu
                85                  90                  95

Leu Gly Val Val Asn His Leu Asp Leu Phe Gly Cys Glu Tyr Leu Thr
            100                 105                 110

Asp Lys Asn Leu Leu Ala Tyr Leu Asp Ile Leu Gln His Leu Ser Ser
        115                 120                 125

Tyr Met Lys Arg Thr Ile Phe His Ser Leu Leu Leu Tyr Tyr Ala Ser
    130                 135                 140

Lys Ala Phe Leu Phe Trp Ile Met Ala Arg Pro Lys Glu Tyr Val Lys
145                 150                 155                 160

Ile Tyr Asn Asn Leu Ile Ser Ser Asp Tyr Asn Ser Pro Ser Ser Ser
                165                 170                 175

Ser Asp Asn Gly Gly Ser Asn Asn Ser Asp Lys Thr Ser Ile Ser Gln
            180                 185                 190

Leu Val Ser Leu Leu Phe Asp Asp Val Tyr Ser Thr Phe Ser Gly Ser
        195                 200                 205

Ser Leu Leu Thr Asn Val Asn Asn Asp His His Tyr His Leu His His
    210                 215                 220

Ser Ser Ser Ser Ser Lys Thr Thr Asn Thr Asn Ser Pro Asn Ser Ile
225                 230                 235                 240
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Ser Lys Thr Ser Ile Lys Gln Ser Ser Val Asn Ala Ser Gly Asn Val
245 250 255

Ser Pro Ser Gln Phe Ser Thr Gly Asn Asp Ala Ser Pro Thr Ser Pro
260 265 270

Met Ala Ser Leu Ser Ser Pro Leu Asn Thr Asn Ile Leu Gly Tyr Pro
275 280 285

Leu Ser Pro Ile Thr Ser Thr Leu Gly Gln Ala Asn Thr Ser Thr Ser
290 295 300

Thr Thr Ala Ala Thr Thr Lys Thr Asp Ala Asp Thr Pro Ser Thr Met
305 310 315 320

Asn Thr Asn Asn Asn Asn Asn Asn Asn Asn Ser Ala Asn Leu Asn Asn
325 330 335

Ile Pro Gln Arg Ile Phe Ser Leu Asp Asp Ile Ser Ser Phe Asn Ser
340 345 350

Ser Arg Lys Ser Leu Asn Leu Asp Asp Ser Asn Ser Leu Phe Leu Trp
355 360 365

Asp Thr Ser Gln His Ser Asn Ala Ser Met Thr Asn Thr Asn Met His
370 375 380

Ala Gly Val Asn Asn Ser Gln Ser Gln Asn Asp Gln Ser Ser Leu Asn
385 390 395 400

Tyr Met Glu Asn Ile Met Glu Leu Tyr Ser Asn Tyr Thr Gly Ser Glu
405 410 415

Leu Ser Ser His Thr Ala Ile Leu Arg Phe Leu Val Val Leu Thr Leu
420 425 430

Leu Asp Ser Glu Val Tyr Asp Glu Met Asn Ser Asn Ser Tyr Arg Lys
435 440 445

Ile Ser Glu Pro Ile Met Asn Ile Asn Pro Lys Asp Ser Asn Thr Ser
450 455 460

Ser Trp Gly Ser Ala Ser Lys Asn Pro Ser Ile Arg His Leu Thr His
465 470 475 480

Gly Leu Lys Lys Leu Thr Leu Gln Gln Gly Arg Lys Arg Asn Val Lys
485 490 495

Phe Leu Thr Tyr Leu Ile Arg Asn Leu Asn Gly Gly Gln Phe Val Ser
500 505 510

Asp Val Ser Leu Ile Asp Ser Ile Arg Ser Ile Leu Phe Leu Met Thr
515 520 525

Met Thr Ser Ser Ile Ser Gln Ile Asp Ser Asn Ile Ala Ser Val Ile
530 535 540

Phe Ser Lys Arg Phe Tyr Asn Leu Leu Gly Gln Asn Leu Glu Val Gly
545 550 555 560

Thr Asn Trp Asn Ser Ala Thr Ala Asn Thr Phe Ile Ser His Cys Val
565 570 575

Glu Arg Asn Pro Leu Thr His Arg Arg Leu Gln Leu Glu Phe Phe Ala
580 585 590

Ser Gly Leu Gln Leu Asp Ser Asp Leu Phe Leu Arg His Leu Gln Leu
595 600 605

Glu Lys Glu Leu Asn His Ile Asp Leu Pro Lys Ile Ser Leu Tyr Thr
610 615 620

Glu Gly Phe Arg Val Phe Phe His Leu Val Ser Thr Lys Lys Leu His
625 630 635 640

Glu Asp Ile Ala Glu Lys Thr Ser Ser Val Leu Lys Arg Leu Phe Cys
645 650 655

Ile Ile Ala Asp Ile Leu Leu Lys Ala Thr Pro Tyr Phe Asp Asp Asn
660 665 670

Val Thr Lys Ile Ile Ala Ser Ile Leu Asp Gly His Ile Leu Asp Gln
675 680 685

Phe Asp Ala Ala Arg Thr Leu Ser Asn Asp Asp His Val Ser Phe Asp
690 695 700

Ala Ala Thr Ser Val Tyr Thr Glu Pro Thr Glu Ile Ile His Asn Ser
705 710 715 720

Ser Asp Ala Ser Leu Val Ser Ser Leu Ser Gln Ser Pro Leu Ser Ile
725 730 735

Asn Ser Gly Ser Asn Ile Thr Asn Thr Arg Thr Trp Asp Ile Gln Ser
740 745 750

Ile Leu Pro Thr Leu Ser Asn Arg Ser Ser Ala Ser Asp Leu Ser Leu
755 760 765

Ser Asn Ile Leu Thr Asn Pro Leu Glu Ala Gln Gln Asn Asn Asn Ala
770 775 780

Asn Leu Leu Ala His Arg Leu Ser Gly Val Pro Thr Thr Lys Arg Tyr
785 790 795 800

Ala Ser Pro Asn Asp Ser Glu Arg Ser Arg Gln Ser Pro Tyr Ser Ser
805 810 815

Pro Pro Gln Leu Gln Gln Ser Asp Leu Pro Ser Pro Leu Ser Val Leu
820 825 830

Ser Ser Ser Ala Gly Phe Ser Ser Asn His Ser Ile Thr Ala Thr Pro
835 840 845

Thr Ile Leu Lys Asn Ile Lys Ser Pro Lys Pro Asn Lys Thr Lys Lys
850 855 860

Ile Ala Asp Asp Lys Gln Leu Lys Gln Pro Ser Tyr Ser Arg Val Ile
865 870 875 880

Leu Ser Asp Asn Asp Glu Ala Arg Lys Ile Met Met Asn Ile Phe Ser
885 890 895

Ile Phe Lys Arg Met Thr Asn Trp Phe Ile Arg Pro Asp Ala Asn Thr
900 905 910

Glu Phe Pro Lys Thr Phe Thr Asp Ile Ile Lys Pro Leu Phe Val Ser
915 920 925

Ile Leu Asp Ser Asn Gln Arg Leu Gln Val Thr Ala Arg Ala Phe Ile
930 935 940

Glu Ile Pro Leu Ser Tyr Ile Ala Thr Phe Glu Asp Ile Asp Asn Asp
945 950 955 960

Leu Asp Pro Arg Val Leu Asn Asp His Tyr Leu Leu Cys Thr Tyr Ala
965 970 975

Val Thr Leu Phe Ala Ser Ser Leu Phe Asp Leu Lys Leu Glu Asn Ala
980 985 990

Lys Arg Glu Met Leu Leu Asp Ile Ile Val Lys Phe Gln Arg Val Arg
995 1000 1005

Ser Tyr Leu Ser Asn Leu Ala Glu Lys His Asn Leu Val Gln Ala Ile
1010 1015 1020

Ile Thr Thr Glu Arg Leu Thr Leu Pro Leu Leu Val Gly Ala Val Gly
1025 1030 1035 1040

Ser Gly Ile Phe Ile Ser Leu Tyr Cys Ser Arg Gly Asn Thr Pro Arg
1045 1050 1055

Leu Ile Lys Ile Ser Cys Cys Glu Phe Leu Arg Ser Leu Arg Phe Tyr
1060 1065 1070

Gln Lys Tyr Val Gly Ala Leu Asp Gln Tyr Ser Ile Tyr Asn Ile Asp
1075 1080 1085

Phe Ile Asp Ala Met Ala Gln Asp Asn Phe Thr Ala Ser Gly Ser Val
1090 1095 1100

Ala Leu Gln Arg Arg Leu Arg Asn Asn Ile Leu Thr Tyr Ile Lys Gly
1105 1110 1115 1120

Ser Asp Ser Ile Leu Leu Asp Ser Met Asp Val Ile Tyr Lys Lys Trp
1125 1130 1135

Phe Tyr Phe Ser Cys Ser Lys Ser Val Thr Gln Glu Glu Leu Val Asp
1140 1145 1150

Phe Arg Ser Leu Ala Gly Ile Leu Ala Ser Met Ser Gly Ile Leu Ser
1155 1160 1165

Asp Met Gln Glu Leu Glu Lys Ser Lys Ser Ala Pro Asp Asn Glu Gly
1170 1175 1180

Asp Ser Leu Ser Phe Glu Ser Arg Asn Pro Ala Tyr Glu Val His Lys
1185 1190 1195 1200

Ser Leu Lys Leu Glu Leu Thr Lys Lys Met Asn Phe Phe Ile Ser Lys
1205 1210 1215

Gln Cys Gln Trp Leu Asn Asn Pro Asn Leu Leu Thr Arg Glu Asn Ser
1220 1225 1230

Arg Asp Ile Leu Ser Ile Glu Leu His Pro Leu Ser Phe Asn Leu Leu
1235 1240 1245

Phe Asn Asn Leu Gly Leu Lys Ile Asp Glu Leu Met Ser Ile Asp Leu
1250 1255 1260

Ser Lys Ser His Glu Asp Ser Ser Phe Val Leu Leu Glu Gln Ile Ile
1265 1270 1275 1280

Ile Ile Ile Arg Thr Ile Leu Lys Arg Asp Asp Asp Glu Lys Ile Met
1285 1290 1295

Leu Leu Phe Ser Thr Asp Leu Leu Asp Ala Val Asp Lys Leu Ile Glu
1300 1305 1310

Ile Val Glu Lys Ile Ser Ile Lys Ser Ser Lys Tyr Tyr Lys Gly Ile
1315 1320 1325

Ile Gln Met Ser Lys Met Phe Arg Ala Phe Glu His Ser Glu Lys Asn
1330 1335 1340

Leu Gly Ile Ser Asn His Phe His Leu Lys Asn Lys Trp Leu Lys Leu
1345 1350 1355 1360

Val Ile Gly Trp Phe Lys Leu Ser Ile Asn Lys Asp Tyr Asp Phe Glu
1365 1370 1375

Asn Leu Ser Arg Pro Leu Arg Glu Met Asp Leu Gln Lys Arg Asp Glu
1380 1385 1390

Asp Phe Leu Tyr Ile Asp Thr Ser Ile Glu Ser Ala Lys Ala Leu Ala
1395 1400 1405

Tyr Leu Thr His Asn Val Pro Leu Glu Ile Pro Pro Ser Ser Ser Lys
1410 1415 1420

Glu Asp Trp Asn Arg Ser Ser Thr Val Ser Phe Gly Asn His Phe Thr
1425 1430 1435 1440

Ile Leu Leu Lys Gly Leu Glu Lys Ser Ala Asp Leu Asn Gln Phe Pro
1445 1450 1455

Val Ser Leu Arg His Lys Ile Ser Ile Leu Asn Glu Asn Val Ile Ile
1460 1465 1470

Ala Leu Thr Asn Leu Ser Asn Ala Asn Val Asn Val Ser Leu Lys Phe
1475 1480 1485

Thr Leu Pro Met Gly Tyr Ser Pro Asn Lys Asp Ile Arg Ile Ala Phe
1490 1495 1500

Leu Arg Val Phe Ile Asp Ile Val Thr Asn Tyr Pro Val Asn Pro Glu
1505 1510 1515 1520

Lys His Glu Met Asp Lys Met Leu Ala Ile Asp Asp Phe Leu Lys Tyr
1525 1530 1535

Ile Ile Lys Asn Pro Ile Leu Ala Phe Phe Gly Ser Leu Ala Cys Ser
1540 1545 1550

Pro Ala Asp Val Asp Leu Tyr Ala Gly Gly Phe Leu Asn Ala Phe Asp
1555 1560 1565

Thr Arg Asn Ala Ser His Ile Leu Val Thr Glu Leu Leu Lys Gln Glu
1570 1575 1580

Ile Lys Arg Ala Ala Arg Ser Asp Asp Ile Leu Arg Arg Asn Ser Cys
1585 1590 1595 1600

Ala Thr Arg Ala Leu Ser Leu Tyr Thr Arg Ser Arg Gly Asn Lys Tyr
1605 1610 1615

Leu Ile Lys Thr Leu Arg Pro Val Leu Gln Gly Ile Val Asp Asn Lys
1620 1625 1630

Glu Ser Phe Glu Ile Asp Lys Met Lys Pro Gly Ser Glu Asn Ser Glu
1635 1640 1645

Lys Met Leu Asp Leu Phe Glu Lys Tyr Met Thr Arg Leu Ile Asp Ala
1650 1655 1660

Ile Thr Ser Ser Ile Asp Asp Phe Pro Ile Glu Leu Val Asp Ile Cys
1665 1670 1675 1680

Lys Thr Ile Tyr Asn Ala Ala Ser Val Asn Phe Pro Glu Tyr Ala Tyr
1685 1690 1695

Ile Ala Val Gly Ser Phe Val Phe Leu Arg Phe Ile Gly Pro Ala Leu
1700 1705 1710

Val Ser Pro Asp Ser Glu Asn Ile Ile Ile Val Thr His Ala His Asp
1715 1720 1725

Arg Lys Pro Phe Ile Thr Leu Ala Lys Val Ile Gln Ser Leu Ala Asn
1730 1735 1740

Gly Arg Glu Asn Ile Phe Lys Lys Asp Ile Leu Val Ser Lys Glu Glu
1745 1750 1755 1760

Phe Leu Lys Thr Cys Ser Asp Lys Ile Phe Asn Phe Leu Ser Glu Leu
1765 1770 1775

Cys Lys Ile Pro Thr Asn Asn Phe Thr Val Asn Val Arg Glu Asp Pro
1780 1785 1790

Thr Pro Ile Ser Phe Asp Tyr Ser Phe Leu His Lys Phe Phe Tyr Leu
1795 1800 1805

Asn Glu Phe Thr Ile Arg Lys Glu Ile Ile Asn Glu Ser Lys Leu Pro
1810 1815 1820

Gly Glu Phe Ser Phe Leu Lys Asn Thr Val Met Leu Asn Asp Lys Ile
1825 1830 1835 1840

Leu Gly Val Leu Gly Gln Pro Ser Met Glu Ile Lys Asn Glu Ile Pro
1845 1850 1855

Pro Phe Val Val Glu Asn Arg Glu Lys Tyr Pro Ser Leu Tyr Glu Phe
1860 1865 1870

Met Ser Arg Tyr Ala Phe Lys Lys Val Asp Met Lys Glu Glu Glu Glu
1875 1880 1885

Asp Asn Ala Pro Phe Val His Glu Ala Met Thr Leu Asp Gly Ile Gln
1890 1895 1900

Ile Ile Val Val Thr Phe Thr Asn Cys Glu Tyr Asn Asn Phe Val Met
1905 1910 1915 1920

Asp Ser Leu Val Tyr Lys Val Leu Gln Ile Tyr Ala Arg Met Trp Cys
1925 1930 1935

Ser Lys His Tyr Val Val Ile Asp Cys Thr Thr Phe Tyr Gly Gly Lys
1940 1945 1950

Ala Asn Phe Gln Lys Leu Thr Thr Leu Phe Phe Ser Leu Ile Pro Glu
1955 1960 1965

Gln Ala Ser Ser Asn Cys Met Gly Cys Tyr Tyr Phe Asn Val Asn Lys
1970 1975 1980

Ser Phe Met Asp Gln Trp Ala Ser Ser Tyr Thr Val Glu Asn Pro Tyr
1985 1990 1995 2000

Leu Val Thr Thr Ile Pro Arg Cys Phe Ile Asn Ser Asn Thr Asp Gln
2005 2010 2015

Ser Leu Ile Lys Ser Leu Gly Leu Ser Gly Arg Ser Leu Glu Val Leu
2020 2025 2030

Lys Asp Val Arg Val Thr Leu His Asp Ile Thr Leu Tyr Asp Lys Glu
2035 2040 2045

Lys Lys Lys Phe Cys Pro Val Ser Leu Lys Ile Gly Asn Lys Tyr Phe
2050 2055 2060

Gln Val Leu His Glu Ile Pro Gln Leu Tyr Lys Val Thr Val Ser Asn
2065 2070 2075 2080

Arg Thr Phe Ser Ile Lys Phe Asn Asn Val Tyr Lys Ile Ser Asn Leu
2085 2090 2095

Ile Ser Val Asp Val Ser Asn Thr Thr Gly Val Ser Ser Glu Phe Thr
2100 2105 2110

Leu Ser Leu Asp Asn Glu Glu Lys Leu Val Phe Cys Ser Pro Lys Tyr
2115 2120 2125

Leu Glu Ile Val Lys Met Phe Tyr Tyr Ala Gln Leu Lys Met Glu Glu
2130 2135 2140

Asp Phe Gly Thr Asp Phe Ser Asn Asp Ile Ser Phe Ser Thr Ser Ser
2145 2150 2155 2160

Ser Ala Val Asn Ala Ser Tyr Cys Asn Val Lys Glu Val Gly Glu Ile
2165 2170 2175

Ile Ser His Leu Ser Leu Val Ile Leu Val Gly Leu Phe Asn Glu Asp
2180 2185 2190

Asp Leu Val Lys Asn Ile Ser Tyr Asn Leu Leu Val Ala Thr Gln Glu
2195 2200 2205

Ala Phe Asn Leu Asp Phe Gly Thr Arg Leu His Lys Ser Pro Glu Thr
2210 2215 2220

Tyr Val Pro Asp Asp Thr Thr Thr Phe Leu Ala Leu Ile Phe Lys Ala
2225 2230 2235 2240

Phe Ser Glu Ser Ser Thr Glu Leu Thr Pro Tyr Ile Trp Lys Tyr Met
2245 2250 2255

Leu Asp Gly Leu Glu Asn Asp Val Ile Pro Gln Glu His Ile Pro Thr
2260 2265 2270

Val Val Cys Ser Leu Ser Tyr Trp Val Pro Asn Leu Tyr Glu His Val
2275 2280 2285

Tyr Leu Ala Asn Asp Glu Glu Gly Pro Glu Ala Ile Ser Arg Ile Ile
2290 2295 2300

Tyr Ser Leu Ile Arg Leu Thr Val Lys Glu Pro Asn Phe Thr Thr Ala
2305 2310 2315 2320

Tyr Leu Gln Gln Ile Trp Phe Leu Leu Ala Leu Asp Gly Arg Leu Thr
2325 2330 2335

Asn Val Ile Val Glu Glu Ile Val Ser His Ala Leu Asp Arg Asp Ser
2340 2345 2350

Glu Asn Arg Asp Trp Met Lys Ala Val Ser Ile Leu Thr Ser Phe Pro
2355 2360 2365

Thr Thr Glu Ile Ala Cys Gln Val Ile Glu Lys Leu Ile Asn Met Ile
2370 2375 2380

Lys Ser Phe Leu Pro Ser Leu Ala Val Glu Ala Ser Ala His Ser Trp
2385 2390 2395 2400

Ser Glu Leu Thr Ile Leu Ser Lys Ile Ser Val Ser Ile Phe Phe Glu
2405 2410 2415

Ser Pro Leu Leu Ser Gln Met Tyr Leu Pro Glu Ile Leu Phe Ala Val
2420 2425 2430

Ser Leu Leu Ile Asp Val Gly Pro Ser Glu Ile Arg Val Ser Leu Tyr
2435 2440 2445

Glu Leu Leu Met Asn Val Cys His Ser Leu Thr Asn Asn Glu Ser Leu
2450 2455 2460

Pro Glu Arg Asn Arg Lys Asn Leu Asp Ile Val Cys Ala Thr Phe Ala
2465 2470 2475 2480

Arg Gln Lys Leu Asn Phe Ile Ser Gly Phe Ser Gln Glu Lys Gly Arg
2485 2490 2495

Val Leu Pro Asn Phe Ala Ala Ser Ser Phe Ser Ser Lys Phe Gly Thr
2500 2505 2510

Leu Asp Leu Phe Thr Lys Asn Ile Met Leu Leu Met Glu Tyr Gly Ser
2515 2520 2525

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Ile Ser Glu Gly Ala Gln Trp Glu Ala Lys Tyr Lys Lys Tyr Leu Met
    2530                2535                2540

Asp Ala Ile Phe Gly His Arg Ser Phe Phe Ser Ala Arg Ala Met Met
2545                2550                2555                2560

Ile Leu Gly Ile Met Ser Lys Ser His Thr Ser Leu Phe Leu Cys Lys
                2565                2570                2575

Glu Leu Leu Val Glu Thr Met Lys Val Phe Ala Glu Pro Val Val Asp
            2580                2585                2590

Asp Glu Gln Met Phe Ile Ile Ile Ala His Val Phe Thr Tyr Ser Lys
        2595                2600                2605

Ile Val Glu Gly Leu Asp Pro Ser Ser Glu Leu Met Lys Glu Leu Phe
    2610                2615                2620

Trp Leu Ala Thr Ile Cys Val Glu Ser Pro His Pro Leu Leu Phe Glu
2625                2630                2635                2640

Gly Gly Leu Leu Phe Met Val Asn Cys Leu Lys Arg Leu Tyr Thr Val
                2645                2650                2655

His Leu Gln Leu Gly Phe Asp Gly Lys Ser Leu Ala Lys Lys Leu Met
            2660                2665                2670

Glu Ser Arg Asn Phe Ala Ala Thr Leu Leu Ala Lys Leu Glu Ser Tyr
        2675                2680                2685

Asn Gly Cys Ile Trp Asn Glu Asp Asn Phe Pro His Ile Ile Leu Gly
    2690                2695                2700

Phe Ile Ala Asn Gly Leu Ser Ile Pro Val Val Lys Gly Ala Ala Leu
2705                2710                2715                2720

Asp Cys Leu Gln Ala Leu Phe Lys Asn Thr Tyr Tyr Glu Arg Lys Ser
                2725                2730                2735

Asn Pro Lys Ser Ser Asp Tyr Leu Cys Tyr Leu Phe Leu Leu His Leu
            2740                2745                2750

Val Leu Ser Pro Glu Gln Leu Ser Thr Leu Leu Leu Glu Val Gly Phe
        2755                2760                2765

Glu Asp Glu Leu Val Pro Leu Asn Asn Thr Leu Lys Val Pro Leu Thr
    2770                2775                2780

Leu Ile Asn Trp Leu Ser Ser Asp Ser Asp Lys Ser Asn Ile Val Leu
2785                2790                2795                2800

Tyr Gln Gly Ala Leu Leu Phe Ser Cys Val Met Ser Asp Glu Pro Cys
                2805                2810                2815

Lys Phe Arg Phe Ala Leu Leu Met Arg Tyr Leu Leu Lys Val Asn Pro
            2820                2825                2830

Ile Cys Val Phe Arg Phe Tyr Thr Leu Thr Arg Lys Glu Phe Arg Arg
        2835                2840                2845
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Leu Ser Thr Leu Glu Gln Ser Ser Glu Ala Val Ala Val Ser Phe Glu
    2850                2855                2860

Leu Ile Gly Met Leu Val Thr His Ser Glu Phe Asn Tyr Leu Glu Glu
2865                2870                2875                2880

Phe Asn Asp Glu Met Val Glu Leu Leu Lys Lys Arg Gly Leu Ser Val
                2885                2890                2895

Val Lys Pro Leu Asp Ile Phe Asp Gln Glu His Ile Glu Lys Leu Lys
            2900                2905                2910

Gly Glu Gly Glu His Gln Val Ala Ile Tyr Glu Arg Lys Arg Leu Ala
        2915                2920                2925

Thr Met Ile Leu Ala Arg Met Ser Cys Ser
    2930                2935
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3079 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ser Gln Pro Thr Lys Asn Lys Lys Lys Glu His Gly Thr Asp Ser
1 5 10 15

Lys Ser Ser Arg Met Thr Arg Thr Leu Val Asn His Ile Leu Phe Glu
20 25 30

Arg Ile Leu Pro Ile Leu Pro Val Glu Ser Asn Leu Ser Thr Tyr Ser
35 40 45

Glu Val Glu Glu Tyr Ser Ser Phe Ile Ser Cys Arg Ser Val Leu Ile
50 55 60

Asn Val Thr Val Ser Arg Asp Ala Asn Ala Met Val Glu Gly Thr Leu
65 70 75 80

Glu Leu Ile Glu Ser Leu Leu Gln Gly His Glu Ile Ile Ser Asp Lys
85 90 95

Gly Ser Ser Asp Val Ile Glu Ser Ile Leu Ile Ile Leu Arg Leu Leu
100 105 110

Ser Asp Ala Leu Glu Tyr Asn Trp Gln Asn Gln Glu Ser Leu His Tyr
115 120 125

Asn Asp Ile Ser Thr His Val Glu His Asp Gln Glu Gln Lys Tyr Arg
130 135 140

Pro Lys Leu Asn Ser Ile Leu Pro Asp Tyr Ser Ser Thr His Ser Asn
145 150 155 160

Gly Asn Lys His Phe Phe His Gln Ser Lys Pro Gln Ala Leu Ile Pro
165 170 175

Glu Leu Ala Ser Lys Leu Leu Glu Ser Cys Ala Lys Leu Lys Phe Asn
180 185 190

Thr Arg Thr Leu Gln Ile Leu Gln Asn Met Ile Ser His Val His Gly
195 200 205

Asn Ile Leu Thr Thr Leu Ser Ser Ser Ile Leu Pro Arg His Lys Ser
210 215 220

Tyr Leu Thr Arg His Asn His Pro Ser His Cys Lys Met Ile Asp Ser
225 230 235 240

Thr Leu Gly His Ile Leu Arg Phe Val Ala Ala Ser Asn Pro Ser Glu
245 250 255

Tyr Phe Glu Phe Ile Arg Lys Ser Val Gln Val Pro Val Thr Gln Thr
260 265 270

His Thr His Ser His Ser His Ser His Ser Leu Pro Ser Ser Val Tyr
275 280 285

Asn Ser Ile Val Pro His Phe Asp Leu Phe Ser Phe Ile Tyr Leu Ser
290 295 300

Lys His Asn Phe Lys Lys Tyr Leu Glu Leu Ile Lys Asn Leu Ser Val
305 310 315 320

Thr Leu Arg Lys Thr Ile Tyr His Cys Leu Leu Leu His Tyr Ser Ala
325 330 335

Lys Ala Ile Met Phe Trp Ile Met Ala Arg Pro Ala Glu Tyr Tyr Glu
340 345 350

Leu Phe Asn Leu Leu Lys Asp Asn Asn Asn Glu His Ser Lys Ser Leu
355 360 365

Asn Thr Leu Asn His Thr Leu Phe Glu Glu Ile His Ser Thr Phe Asn
370 375 380

Val Asn Ser Met Ile Thr Thr Asn Gln Asn Ala His Gln Gly Ser Ser
385 390 395 400

Ser Pro Ser Ser Ser Ser Pro Ser Ser Pro Pro Ser Ser Ser Ser Ser
405 410 415

Asp Asn Asn Asn Gln Asn Ile Ile Ala Lys Ser Leu Ser Arg Gln Leu
420 425 430

Ser His His Gln Ser Tyr Ile Gln Gln Gln Ser Glu Arg Lys Leu His
435 440 445

Ser Ser Trp Thr Thr Asn Ser Gln Ser Ser Thr Ser Leu Ser Ser Ser
450 455 460

Thr Ser Asn Ser Thr Thr Thr Asp Phe Ser Thr His Thr Gln Pro Gly
465 470 475 480

Glu Tyr Asp Pro Ser Leu Pro Asp Thr Pro Thr Met Ser Asn Ile Thr
485 490 495

Ile Ser Ala Ser Ser Leu Leu Ser Gln Thr Pro Thr Pro Thr Thr Gln
500 505 510

Leu Gln Gln Arg Leu Asn Ser Ala Ala Ala Ala Ala Ala Ala Ala Ala
515 520 525

Ser Pro Ser Asn Ser Thr Pro Thr Gly Tyr Thr Ala Glu Gln Gln Ser
530 535 540

Arg Ala Ser Tyr Asp Ala His Lys Thr Gly His Thr Gly Lys Asp Tyr
545 550 555 560

Asp Glu His Phe Leu Ser Val Thr Arg Leu Asp Asn Val Leu Glu Leu
565 570 575

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Tyr Thr His Phe Asp Asp Thr Glu Val Leu Pro His Thr Ser Val Leu
            580                 585                 590

Lys Phe Leu Thr Thr Leu Thr Met Phe Asp Ile Asp Leu Phe Asn Glu
        595                 600                 605

Leu Asn Ala Thr Ser Phe Lys Tyr Ile Pro Asp Cys Thr Met His Arg
    610                 615                 620

Pro Lys Glu Arg Thr Ser Ser Phe Asn Asn Thr Ala His Glu Thr Gly
625                 630                 635                 640

Ser Glu Lys Thr Ser Gly Ile Lys His Ile Thr Gln Gly Leu Lys Lys
                645                 650                 655

Leu Thr Ser Leu Pro Ser Ser Thr Lys Lys Thr Val Lys Phe Val Lys
            660                 665                 670

Met Leu Leu Arg Asn Leu Asn Gly Asn Gln Ala Val Ser Asp Val Ala
        675                 680                 685

Leu Leu Asp Thr Met Arg Ala Leu Leu Ser Phe Phe Thr Met Thr Ser
    690                 695                 700

Ala Val Phe Leu Val Asp Arg Asn Leu Pro Ser Val Leu Phe Ala Lys
705                 710                 715                 720

Arg Leu Ile Pro Ile Met Gly Thr Asn Leu Ser Val Gly Gln Asp Trp
                725                 730                 735

Asn Ser Lys Ile Asn Asn Ser Leu Met Val Cys Leu Lys Lys Asn Ser
            740                 745                 750

Thr Thr Phe Val Gln Leu Gln Leu Ile Phe Phe Ser Ser Ala Ile Gln
        755                 760                 765

Phe Asp His Glu Leu Leu Leu Ala Arg Leu Ser Ile Asp Thr Met Ala
    770                 775                 780

Asn Asn Leu Asn Met Gln Lys Leu Cys Leu Tyr Thr Glu Gly Phe Arg
785                 790                 795                 800

Ile Phe Phe Asp Ile Pro Ser Lys Lys Glu Leu Arg Lys Ala Ile Ala
                805                 810                 815

Val Lys Ile Ser Lys Phe Phe Lys Thr Leu Phe Ser Ile Ile Ala Asp
            820                 825                 830

Ile Leu Leu Gln Glu Phe Pro Tyr Phe Asp Glu Gln Ile Thr Asp Ile
        835                 840                 845

Val Ala Ser Ile Leu Asp Gly Thr Ile Ile Asn Glu Tyr Gly Thr Lys
    850                 855                 860

Lys His Phe Lys Gly Ser Ser Pro Ser Leu Cys Ser Thr Thr Arg Ser
865                 870                 875                 880

Arg Ser Gly Ser Thr Ser Gln Ser Ser Met Thr Pro Val Ser Pro Leu
                885                 890                 895
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Gly Leu Asp Thr Asp Ile Cys Pro Met Asn Thr Leu Ser Leu Val Gly
900 905 910

Ser Ser Thr Ser Arg Asn Ser Asp Asn Val Asn Ser Leu Asn Ser Ser
915 920 925

Pro Lys Asn Leu Ser Ser Asp Pro Tyr Leu Ser His Leu Val Ala Pro
930 935 940

Arg Ala Arg His Ala Leu Gly Gly Pro Ser Ser Ile Ile Arg Asn Lys
945 950 955 960

Ile Pro Thr Thr Leu Thr Ser Pro Pro Gly Thr Glu Lys Ser Ser Pro
965 970 975

Val Gln Arg Pro Gln Thr Glu Ser Ile Ser Ala Thr Pro Met Ala Ile
980 985 990

Thr Asn Ser Thr Pro Leu Ser Ser Ala Ala Phe Gly Ile Arg Ser Pro
995 1000 1005

Leu Gln Lys Ile Arg Thr Arg Arg Tyr Ser Asp Glu Ser Leu Gly Lys
1010 1015 1020

Phe Met Lys Ser Thr Asn Asn Tyr Ile Gln Glu His Leu Ile Pro Lys
1025 1030 1035 1040

Asp Leu Asn Glu Ala Thr Leu Gln Asp Ala Arg Arg Ile Met Ile Asn
1045 1050 1055

Ile Phe Ser Ile Phe Lys Arg Pro Asn Ser Tyr Phe Ile Ile Pro His
1060 1065 1070

Asn Ile Asn Ser Asn Leu Gln Trp Val Ser Gln Asp Phe Arg Asn Ile
1075 1080 1085

Met Lys Pro Ile Phe Val Ala Ile Val Ser Pro Asp Val Asp Leu Gln
1090 1095 1100

Asn Thr Ala Gln Ser Phe Met Asp Thr Leu Leu Ser Asn Val Ile Thr
1105 1110 1115 1120

Tyr Gly Glu Ser Asp Glu Asn Ile Ser Ile Glu Gly Tyr His Leu Leu
1125 1130 1135

Cys Ser Tyr Thr Val Thr Leu Phe Ala Met Gly Leu Phe Asp Leu Lys
1140 1145 1150

Ile Asn Asn Glu Lys Arg Gln Ile Leu Leu Asp Ile Thr Val Lys Phe
1155 1160 1165

Met Lys Val Arg Ser His Leu Ala Gly Ile Ala Glu Ala Ser His His
1170 1175 1180

Met Glu Tyr Ile Ser Asp Ser Glu Lys Leu Thr Phe Pro Leu Ile Met
1185 1190 1195 1200

Gly Thr Val Gly Arg Ala Leu Phe Val Ser Leu Tyr Ser Ser Gln Gln
1205 1210 1215

Lys Ile Glu Lys Thr Leu Lys Ile Ala Tyr Thr Glu Tyr Leu Ser Ala
1220 1225 1230

Ile Asn Phe His Glu Arg Asn Ile Asp Asp Ala Asp Lys Thr Trp Val
1235 1240 1245

His Asn Ile Glu Phe Val Glu Ala Met Cys His Asp Asn Tyr Thr Thr
1250 1255 1260

Ser Gly Ser Ile Ala Phe Gln Arg Arg Thr Arg Asn Asn Ile Leu Arg
1265 1270 1275 1280

Phe Ala Thr Ile Pro Asn Ala Ile Leu Leu Asp Ser Met Arg Met Ile
1285 1290 1295

Tyr Lys Lys Trp His Thr Tyr Thr His Ser Lys Ser Leu Glu Lys Gln
1300 1305 1310

Glu Arg Asn Asp Phe Arg Asn Phe Ala Gly Ile Leu Ala Ser Leu Ser
1315 1320 1325

Gly Ile Leu Phe Ile Asn Lys Lys Ile Leu Gln Glu Met Tyr Pro Tyr
1330 1335 1340

Leu Leu Asp Thr Val Ser Glu Leu Lys Lys Asn Ile Asp Ser Phe Ile
1345 1350 1355 1360

Ser Lys Gln Cys Gln Trp Leu Asn Tyr Pro Asp Leu Leu Thr Arg Glu
1365 1370 1375

Asn Ser Arg Asp Ile Leu Ser Val Glu Leu His Pro Leu Ser Phe Asn
1380 1385 1390

Leu Leu Phe Asn Asn Leu Arg Leu Lys Leu Lys Glu Leu Ala Cys Ser
1395 1400 1405

Asp Leu Ser Ile Pro Glu Asn Glu Ser Ser Tyr Val Leu Leu Glu Gln
1410 1415 1420

Ile Ile Lys Met Leu Arg Thr Ile Leu Gly Arg Asp Asp Asp Asn Tyr
1425 1430 1435 1440

Val Met Met Leu Phe Ser Thr Glu Ile Val Asp Leu Ile Asp Leu Leu
1445 1450 1455

Thr Asp Glu Ile Lys Lys Ile Pro Ala Tyr Cys Pro Lys Tyr Leu Lys
1460 1465 1470

Ala Ile Ile Gln Met Thr Lys Met Phe Ser Ala Leu Gln His Ser Glu
1475 1480 1485

Val Asn Leu Gly Val Lys Asn His Phe His Val Lys Asn Lys Trp Leu
1490 1495 1500

Arg Gln Ile Thr Asp Trp Phe Gln Val Ser Ile Ala Arg Glu Tyr Asp
1505 1510 1515 1520

Phe Glu Asn Leu Ser Lys Pro Leu Lys Glu Met Asp Leu Val Lys Arg
1525 1530 1535

Asp Met Asp Ile Leu Tyr Ile Asp Thr Ala Ile Glu Ala Ser Thr Ala
1540 1545 1550

Ile Ala Tyr Leu Thr Arg His Thr Phe Leu Glu Ile Pro Pro Ala Ala
1555 1560 1565

Ser Asp Pro Glu Leu Ser Arg Ser Arg Ser Val Ile Phe Gly Phe Tyr
1570 1575 1580

Phe Asn Ile Leu Met Lys Gly Leu Glu Lys Ser Ser Asp Arg Asp Asn
1585 1590 1595 1600

Tyr Pro Val Phe Leu Arg His Lys Met Ser Val Leu Asn Asp Asn Val
1605 1610 1615

Ile Leu Ser Leu Thr Asn Leu Ser Asn Thr Asn Val Asp Ala Ser Leu
1620 1625 1630

Gln Phe Thr Leu Pro Met Gly Tyr Ser Gly Asn Arg Asn Ile Arg Asn
1635 1640 1645

Ala Phe Leu Glu Val Phe Ile Asn Ile Val Thr Asn Tyr Arg Thr Tyr
1650 1655 1660

Thr Ala Lys Thr Asp Leu Gly Lys Leu Glu Ala Ala Asp Lys Phe Leu
1665 1670 1675 1680

Arg Tyr Thr Ile Glu His Pro Gln Leu Ser Ser Phe Gly Ala Ala Val
1685 1690 1695

Cys Pro Ala Ser Asp Ile Asp Ala Tyr Ala Ala Gly Leu Ile Asn Ala
1700 1705 1710

Phe Glu Thr Arg Asn Ala Thr His Ile Val Val Ala Gln Leu Ile Lys
1715 1720 1725

Asn Glu Ile Glu Lys Ser Ser Arg Pro Thr Asp Ile Leu Arg Arg Asn
1730 1735 1740

Ser Cys Ala Thr Arg Ser Leu Ser Met Leu Ala Arg Ser Lys Gly Asn
1745 1750 1755 1760

Glu Tyr Leu Ile Arg Thr Leu Gln Pro Leu Leu Lys Lys Ile Ile Gln
1765 1770 1775

Asn Arg Asp Phe Phe Glu Ile Glu Lys Leu Lys Pro Glu Asp Ser Asp
1780 1785 1790

Ala Glu Arg Gln Ile Glu Leu Phe Val Lys Tyr Met Asn Glu Leu Leu
1795 1800 1805

Glu Ser Ile Ser Asn Ser Val Ser Tyr Phe Pro Pro Pro Leu Phe Tyr
1810 1815 1820

Ile Cys Gln Asn Ile Tyr Lys Val Ala Cys Glu Lys Phe Pro Asp His
1825 1830 1835 1840

Ala Ile Ile Ala Ala Gly Ser Phe Val Phe Leu Arg Phe Phe Cys Pro
1845 1850 1855

Ala Leu Val Ser Pro Asp Ser Glu Asn Ile Ile Asp Ile Ser His Leu
1860 1865 1870

Ser Glu Lys Arg Thr Phe Ile Ser Leu Ala Lys Val Ile Gln Asn Ile
1875 1880 1885

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Ala Asn Gly Ser Glu Asn Phe Ser Arg Trp Pro Ala Leu Cys Ser Gln
    1890                1895                1900

Lys Asp Phe Leu Lys Glu Cys Ser Asp Arg Ile Phe Arg Phe Leu Ala
1905                1910                1915                1920

Glu Leu Cys Arg Thr Asp Arg Thr Ile Asp Ile Gln Val Arg Thr Asp
                1925                1930                1935

Pro Thr Pro Ile Ala Phe Asp Tyr Gln Phe Leu His Ser Phe Val Tyr
            1940                1945                1950

Leu Tyr Gly Leu Glu Val Arg Arg Asn Val Leu Asn Glu Ala Lys His
        1955                1960                1965

Asp Asp Gly Asp Ile Asp Gly Asp Asp Phe Tyr Lys Thr Thr Phe Leu
    1970                1975                1980

Leu Ile Asp Asp Val Leu Gly Gln Leu Gly Gln Pro Lys Met Glu Phe
1985                1990                1995                2000

Ser Asn Glu Ile Pro Ile Tyr Ile Arg Glu His Met Asp Asp Tyr Pro
                2005                2010                2015

Glu Leu Tyr Glu Phe Met Asn Arg His Ala Phe Arg Asn Ile Glu Thr
            2020                2025                2030

Ser Thr Ala Tyr Ser Pro Ser Val His Glu Ser Thr Ser Ser Glu Gly
        2035                2040                2045

Ile Pro Ile Ile Thr Leu Thr Met Ser Asn Phe Ser Asp Arg His Val
    2050                2055                2060

Asp Ile Asp Thr Val Ala Tyr Lys Phe Leu Gln Ile Tyr Ala Arg Ile
2065                2070                2075                2080

Trp Thr Thr Lys His Cys Leu Ile Ile Asp Cys Thr Glu Phe Asp Glu
                2085                2090                2095

Gly Gly Leu Asp Met Arg Lys Phe Ile Ser Leu Val Met Gly Leu Leu
            2100                2105                2110

Pro Glu Val Ala Pro Lys Asn Cys Ile Gly Cys Tyr Tyr Phe Asn Val
        2115                2120                2125

Asn Glu Thr Phe Met Asp Asn Tyr Gly Lys Cys Leu Asp Lys Asp Asn
    2130                2135                2140

Val Tyr Val Ser Ser Lys Ile Pro His Tyr Phe Ile Asn Ser Asn Ser
2145                2150                2155                2160

Asp Glu Gly Leu Met Lys Ser Val Gly Ile Thr Gly Gln Gly Leu Lys
                2165                2170                2175

Val Leu Gln Asp Ile Arg Val Ser Leu His Asp Ile Thr Leu Tyr Asp
            2180                2185                2190

Glu Lys Arg Asn Arg Phe Thr Pro Val Ser Leu Lys Ile Gly Asp Ile
        2195                2200                2205
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Tyr Phe Gln Val Leu His Glu Thr Pro Arg Gln Tyr Lys Ile Arg Asp
2210 2215 2220

Met Gly Thr Leu Phe Asp Val Lys Phe Asn Asp Val Tyr Glu Ile Ser
2225 2230 2235 2240

Arg Ile Phe Glu Val His Val Ser Ser Ile Thr Gly Val Ala Ala Glu
2245 2250 2255

Phe Thr Val Thr Phe Gln Asp Glu Arg Arg Leu Ile Phe Ser Ser Pro
2260 2265 2270

Lys Tyr Leu Glu Ile Val Lys Met Phe Tyr Tyr Ala Gln Ile Arg Leu
2275 2280 2285

Glu Ser Glu Tyr Glu Met Asp Asn Asn Ser Ser Thr Ser Ser Pro Asn
2290 2295 2300

Ser Asn Asn Lys Val Lys Gln Gln Lys Glu Arg Thr Ile Leu Leu Cys
2305 2310 2315 2320

His Leu Leu Leu Val Ser Leu Ile Gly Leu Phe Asp Glu Ser Lys Lys
2325 2330 2335

Met Lys Asn Ser Ser Tyr Asn Leu Ile Ala Ala Thr Glu Ala Ser Phe
2340 2345 2350

Gly Leu Asn Phe Gly Ser His Phe His Arg Ser Pro Glu Val Tyr Val
2355 2360 2365

Pro Glu Asp Thr Thr Thr Phe Leu Gly Val Ile Gly Lys Ser Leu Ala
2370 2375 2380

Glu Ser Asn Pro Glu Leu Thr Ala Tyr Met Phe Ile Tyr Val Leu Glu
2385 2390 2395 2400

Ala Leu Lys Asn Asn Val Ile Pro His Val Tyr Ile Pro His Thr Ile
2405 2410 2415

Cys Gly Leu Ser Tyr Trp Ile Pro Asn Leu Tyr Gln His Val Tyr Leu
2420 2425 2430

Ala Asp Asp Glu Glu Gly Pro Glu Asn Ile Ser His Ile Phe Arg Ile
2435 2440 2445

Leu Ile Arg Leu Ser Val Arg Glu Thr Asp Phe Lys Ala Val Tyr Met
2450 2455 2460

Gln Tyr Val Trp Leu Leu Leu Leu Asp Asp Gly Arg Leu Thr Asp Ile
2465 2470 2475 2480

Ile Val Asp Glu Val Ile Asn His Ala Leu Glu Arg Asp Ser Glu Asn
2485 2490 2495

Arg Asp Trp Lys Lys Thr Ile Ser Leu Leu Thr Val Leu Pro Thr Thr
2500 2505 2510

Glu Val Ala Asn Asn Ile Ile Gln Lys Ile Leu Ala Lys Ile Arg Ser
2515 2520 2525

Phe Leu Pro Ser Leu Lys Leu Glu Ala Met Thr Gln Ser Trp Ser Glu
2530 2535 2540

Leu Thr Ile Leu Val Lys Ile Ser Ile His Val Phe Phe Glu Thr Ser
2545 2550 2555 2560

Leu Leu Val Gln Met Tyr Leu Pro Glu Ile Leu Phe Ile Val Ser Leu
2565 2570 2575

Leu Ile Asp Val Gly Pro Arg Glu Leu Arg Ser Ser Leu His Gln Leu
2580 2585 2590

Leu Met Asn Val Cys His Ser Leu Ala Ile Asn Ser Ala Leu Pro Gln
2595 2600 2605

Asp His Arg Asn Asn Leu Asp Glu Ile Ser Asp Ile Phe Ala His Gln
2610 2615 2620

Lys Val Lys Phe Met Phe Gly Phe Ser Glu Asp Lys Gly Arg Ile Leu
2625 2630 2635 2640

Gln Ile Phe Ser Ala Ser Ser Phe Ala Ser Lys Phe Asn Ile Leu Asp
2645 2650 2655

Phe Phe Ile Asn Asn Ile Leu Leu Leu Met Glu Tyr Ser Ser Thr Tyr
2660 2665 2670

Glu Ala Asn Val Trp Lys Thr Arg Tyr Lys Lys Tyr Val Leu Glu Ser
2675 2680 2685

Val Phe Thr Ser Asn Ser Phe Leu Ser Ala Arg Ser Ile Met Ile Val
2690 2695 2700

Gly Ile Met Gly Lys Ser Tyr Ile Thr Glu Gly Leu Cys Lys Ala Met
2705 2710 2715 2720

Leu Ile Glu Thr Met Lys Val Ile Ala Glu Pro Lys Ile Thr Asp Glu
2725 2730 2735

His Leu Phe Leu Ala Ile Ser His Ile Phe Thr Tyr Ser Lys Ile Val
2740 2745 2750

Glu Gly Leu Asp Pro Asn Leu Asp Leu Met Lys His Leu Phe Trp Phe
2755 2760 2765

Ser Thr Leu Phe Leu Glu Ser Arg His Pro Ile Ile Phe Glu Gly Ala
2770 2775 2780

Leu Leu Phe Val Ser Asn Cys Ile Arg Arg Leu Tyr Met Ala Gln Phe
2785 2790 2795 2800

Glu Asn Glu Ser Glu Thr Ser Leu Ile Ser Thr Leu Leu Lys Gly Arg
2805 2810 2815

Lys Phe Ala His Thr Phe Leu Ser Lys Ile Glu Asn Leu Ser Gly Ile
2820 2825 2830

Val Trp Asn Glu Asp Asn Phe Thr His Ile Leu Ile Phe Ile Ile Asn
2835 2840 2845

Lys Gly Leu Ser Asn Pro Phe Ile Lys Ser Thr Ala Phe Asp Phe Leu
2850 2855 2860

Lys Met Met Phe Arg Asn Ser Tyr Phe Glu His Gln Ile Asn Gln Lys
2865 2870 2875 2880

Ser Asp His Tyr Leu Cys Tyr Met Phe Leu Leu Tyr Phe Val Leu Asn
2885 2890 2895

Cys Asn Gln Phe Glu Glu Leu Leu Gly Asp Val Asp Phe Glu Gly Glu
2900 2905 2910

Met Val Asn Ile Glu Asn Lys Asn Thr Ile Pro Lys Ile Leu Leu Glu
2915 2920 2925

Trp Leu Ser Ser Asp Asn Glu Asn Ala Asn Ile Thr Leu Tyr Gln Gly
2930 2935 2940

Ala Ile Leu Phe Lys Cys Ser Val Thr Asp Glu Pro Ser Arg Phe Arg
2945 2950 2955 2960

Phe Ala Leu Ile Ile Arg His Leu Leu Thr Lys Lys Pro Ile Cys Ala
2965 2970 2975

Leu Arg Phe Tyr Ser Val Ile Arg Asn Glu Ile Arg Lys Ile Ser Ala
2980 2985 2990

Phe Glu Gln Asn Ser Asp Cys Val Pro Leu Ala Phe Asp Ile Leu Asn
2995 3000 3005

Leu Leu Val Thr His Ser Glu Ser Asn Ser Leu Glu Lys Leu His Glu
3010 3015 3020

Glu Ser Ile Glu Arg Leu Thr Lys Arg Gly Leu Ser Ile Val Thr Ser
3025 3030 3035 3040

Ser Gly Ile Phe Ala Lys Asn Ser Asp Met Met Ile Pro Leu Asp Val
3045 3050 3055

Lys Pro Glu Asp Ile Tyr Glu Arg Lys Arg Ile Met Thr Met Ile Leu
3060 3065 3070

Ser Arg Met Ser Cys Ser Ala
3075

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 870 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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Met Lys Gly Trp Tyr His Gly Lys Leu Asp Arg Thr Ile Ala Glu Glu
1               5                  10                  15

Arg Leu Arg Gln Ala Gly Lys Ser Gly Ser Tyr Leu Ile Arg Glu Ser
            20                  25                  30

Asp Arg Arg Pro Gly Ser Phe Val Leu Ser Phe Leu Ser Gln Met Asn
        35                  40                  45

Val Val Asn His Phe Arg Ile Ile Ala Met Cys Gly Asp Tyr Tyr Ile
    50                  55                  60

Gly Gly Arg Arg Phe Ser Ser Leu Ser Asp Leu Ile Gly Tyr Tyr Ser
65                  70                  75                  80

His Val Ser Cys Leu Leu Lys Gly Glu Lys Leu Leu Tyr Pro Val Ala
                85                  90                  95

Pro Pro Glu Pro Val Glu Asp Arg Arg Arg Val Arg Ala Ile Leu Pro
            100                 105                 110

Tyr Thr Lys Val Pro Asp Thr Asp Glu Ile Ser Phe Leu Lys Gly Asp
        115                 120                 125

Met Phe Ile Val His Asn Glu Leu Glu Asp Gly Trp Met Trp Val Thr
    130                 135                 140

Asn Leu Arg Thr Asp Glu Gln Gly Leu Ile Val Glu Asp Leu Val Glu
145                 150                 155                 160

Glu Val Gly Arg Glu Glu Asp Pro His Glu Gly Lys Ile Trp Phe His
                165                 170                 175

Gly Lys Ile Ser Lys Gln Glu Ala Tyr Asn Leu Leu Met Thr Val Gly
            180                 185                 190

Gln Val Cys Ser Phe Leu Val Arg Pro Ser Asp Asn Thr Pro Gly Asp
        195                 200                 205

Tyr Ser Leu Tyr Phe Arg Thr Asn Glu Asn Ile Gln Arg Phe Lys Ile
    210                 215                 220

Cys Pro Thr Pro Asn Asn Gln Phe Met Met Gly Gly Arg Tyr Tyr Asn
225                 230                 235                 240

Ser Ile Gly Asp Ile Ile Asp His Tyr Arg Lys Glu Gln Ile Val Glu
                245                 250                 255

Gly Tyr Tyr Leu Lys Glu Pro Val Pro Met Gln Asp Gln Glu Gln Val
            260                 265                 270

Leu Asn Asp Thr Val Asp Gly Lys Glu Ile Tyr Asn Thr Ile Arg Arg
        275                 280                 285

Lys Thr Lys Asp Ala Phe Tyr Lys Asn Ile Val Lys Lys Gly Tyr Leu
    290                 295                 300

Leu Lys Lys Gly Lys Gly Lys Arg Trp Lys Asn Leu Tyr Phe Ile Leu
305                 310                 315                 320
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Glu Gly Ser Asp Ala Gln Leu Ile Tyr Phe Glu Ser Glu Lys Arg Ala
325 330 335

Thr Lys Pro Lys Gly Leu Ile Asp Leu Ser Val Cys Ser Val Tyr Val
340 345 350

Val His Asp Ser Leu Phe Gly Arg Pro Asn Cys Phe Gln Ile Val Val
355 360 365

Gln His Phe Ser Glu Glu His Tyr Ile Phe Tyr Phe Ala Gly Glu Thr
370 375 380

Pro Glu Gln Ala Glu Asp Trp Met Lys Gly Leu Gln Ala Phe Cys Asn
385 390 395 400

Leu Arg Lys Ser Ser Pro Gly Thr Ser Asn Lys Arg Leu Arg Gln Val
405 410 415

Ser Ser Leu Val Leu His Ile Glu Glu Ala His Lys Leu Pro Val Lys
420 425 430

His Phe Thr Asn Pro Tyr Cys Asn Ile Tyr Leu Asn Ser Val Gln Val
435 440 445

Ala Lys Thr His Ala Arg Glu Gly Gln Asn Pro Val Trp Ser Glu Glu
450 455 460

Phe Val Phe Asp Asp Leu Pro Pro Asp Ile Asn Arg Phe Glu Ile Thr
465 470 475 480

Leu Ser Asn Lys Thr Lys Lys Ser Lys Asp Pro Asp Ile Leu Phe Met
485 490 495

Arg Cys Gln Leu Ser Arg Leu Gln Lys Gly His Ala Thr Asp Glu Trp
500 505 510

Phe Leu Leu Ser Ser His Ile Pro Leu Lys Gly Ile Glu Pro Gly Ser
515 520 525

Leu Arg Val Arg Ala Arg Tyr Ser Met Glu Lys Ile Met Pro Glu Glu
530 535 540

Glu Tyr Ser Glu Phe Lys Glu Leu Ile Leu Gln Lys Glu Leu His Val
545 550 555 560

Val Tyr Ala Leu Ser His Val Cys Gly Gln Asp Arg Thr Leu Leu Ala
565 570 575

Ser Ile Leu Leu Arg Ile Phe Leu His Glu Lys Leu Glu Ser Leu Leu
580 585 590

Leu Cys Thr Leu Asn Asp Arg Glu Ile Ser Met Glu Asp Glu Ala Thr
595 600 605

Thr Leu Phe Arg Ala Thr Thr Leu Ala Ser Thr Leu Met Glu Gln Tyr
610 615 620

Met Lys Ala Thr Ala Thr Gln Phe Val His His Ala Leu Lys Asp Ser
625 630 635 640

Ile Leu Lys Ile Met Glu Ser Lys Gln Ser Cys Glu Leu Ser Pro Ser
645 650 655

Lys Leu Glu Lys Asn Glu Asp Val Asn Thr Asn Leu Thr His Leu Leu
660 665 670

Asn Ile Leu Ser Glu Leu Val Glu Lys Ile Phe Met Ala Ser Glu Ile
675 680 685

Leu Pro Pro Thr Leu Arg Tyr Ile Tyr Gly Cys Leu Gln Lys Ser Val
690 695 700

Gln His Lys Trp Pro Thr Asn Thr Thr Met Arg Thr Arg Val Val Ser
705 710 715 720

Gly Phe Val Phe Leu Arg Leu Ile Cys Pro Ala Ile Leu Asn Pro Arg
725 730 735

Met Phe Asn Ile Ile Ser Asp Ser Pro Ser Pro Ile Ala Ala Arg Thr
740 745 750

Leu Ile Leu Val Ala Lys Ser Val Gln Asn Leu Ala Asn Leu Val Glu
755 760 765

Phe Gly Ala Lys Glu Pro Tyr Met Glu Gly Val Asn Pro Phe Ile Lys
770 775 780

Ser Asn Lys His Arg Met Ile Met Phe Leu Asp Glu Leu Gly Asn Val
785 790 795 800

Pro Glu Leu Pro Asp Thr Thr Glu His Ser Arg Thr Asp Leu Ser Arg
805 810 815

Asp Leu Ala Ala Leu His Glu Ile Cys Val Ala His Ser Asp Glu Leu
820 825 830

Arg Thr Leu Ser Asn Glu Arg Gly Ala Gln Gln His Val Leu Lys Lys
835 840 845

Leu Leu Ala Ile Thr Glu Leu Leu Gln Gln Lys Gln Asn Gln Tyr Thr
850 855 860

Lys Thr Asn Asp Val Arg
865 870

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 766 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Schizosaccharomyces pombe

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Thr Lys Arg His Ser Gly Thr Leu Ser Ser Ser Val Leu Pro Gln
1 5 10 15

Thr Asn Arg Leu Ser Leu Leu Arg Asn Arg Glu Ser Thr Ser Val Leu
20 25 30

Tyr Thr Ile Asp Leu Asp Met Glu Ser Asp Val Glu Asp Ala Phe Phe
35 40 45

His Leu Asp Arg Glu Leu His Asp Leu Lys Gln Gln Ile Ser Ser Gln
50 55 60

Ser Lys Gln Asn Phe Val Leu Glu Arg Asp Val Arg Tyr Leu Asp Ser
65 70 75 80

Lys Ile Ala Leu Leu Ile Gln Asn Arg Met Ala Gln Glu Glu Gln His
85 90 95

Glu Phe Ala Lys Arg Leu Asn Asp Asn Tyr Asn Ala Val Lys Gly Ser
100 105 110

Phe Pro Asp Asp Arg Lys Leu Gln Leu Tyr Gly Ala Leu Phe Phe Leu
115 120 125

Leu Gln Ser Glu Pro Ala Tyr Ile Ala Ser Leu Val Arg Arg Val Lys
130 135 140

Leu Phe Asn Met Asp Ala Leu Leu Gln Ile Val Met Phe Asn Ile Tyr
145 150 155 160

Gly Asn Gln Tyr Glu Ser Arg Glu Glu His Leu Leu Leu Ser Leu Phe
165 170 175

Gln Met Val Leu Thr Thr Glu Phe Glu Ala Thr Ser Asp Val Leu Ser
180 185 190

Leu Leu Arg Ala Asn Thr Pro Val Ser Arg Met Leu Thr Thr Tyr Thr
195 200 205

Arg Arg Gly Pro Gly Gln Ala Tyr Leu Arg Ser Ile Leu Tyr Gln Cys
210 215 220

Ile Asn Asp Val Ala Ile His Pro Asp Leu Gln Leu Asp Ile His Pro
225 230 235 240

Leu Ser Val Tyr Arg Tyr Leu Val Asn Thr Gly Gln Leu Ser Pro Ser
245 250 255

Glu Asp Asp Asn Leu Leu Thr Asn Glu Glu Val Ser Glu Phe Pro Ala
260 265 270

Val Lys Asn Ala Ile Gln Glu Arg Ser Ala Gln Leu Leu Leu Leu Thr
275 280 285

Lys Arg Phe Leu Asp Ala Val Leu Asn Ser Ile Asp Glu Ile Pro Tyr
290 295 300

Gly Ile Arg Trp Val Cys Lys Leu Ile Arg Asn Leu Thr Asn Arg Leu
305 310 315 320

Phe Pro Ser Ile Ser Asp Ser Thr Ile Cys Ser Leu Ile Gly Gly Phe
325 330 335

Phe Phe Leu Arg Phe Val Asn Pro Ala Ile Ile Ser Pro Gln Thr Ser
340 345 350

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Met Leu Leu Asp Ser Cys Pro Ser Asp Asn Val Arg Lys Thr Leu Ala
        355                 360                 365

Thr Ile Ala Lys Ile Ile Gln Ser Val Ala Asn Gly Thr Ser Ser Thr
    370                 375                 380

Lys Thr His Leu Asp Val Ser Phe Gln Pro Met Leu Lys Glu Tyr Glu
385                 390                 395                 400

Glu Lys Val His Asn Leu Leu Arg Lys Leu Gly Asn Val Gly Asp Phe
                405                 410                 415

Phe Glu Ala Leu Glu Leu Asp Gln Tyr Ile Ala Leu Ser Lys Lys Ser
            420                 425                 430

Leu Ala Leu Glu Met Thr Val Asn Glu Ile Tyr Leu Thr His Glu Ile
        435                 440                 445

Ile Leu Glu Asn Leu Asp Asn Leu Tyr Asp Pro Asp Ser His Val His
    450                 455                 460

Leu Ile Leu Gln Glu Leu Gly Glu Pro Cys Lys Ser Val Pro Gln Glu
465                 470                 475                 480

Asp Asn Cys Leu Val Thr Leu Pro Leu Tyr Asn Arg Trp Asp Ser Ser
                485                 490                 495

Ile Pro Asp Leu Lys Gln Asn Leu Lys Val Thr Arg Glu Asp Ile Leu
            500                 505                 510

Tyr Val Asp Ala Lys Thr Leu Phe Ile Gln Leu Leu Arg Leu Leu Pro
        515                 520                 525

Ser Gly His Pro Ala Thr Arg Val Pro Leu Asp Leu Pro Leu Ile Ala
    530                 535                 540

Asp Ser Val Ser Ser Leu Lys Ser Met Ser Leu Met Lys Lys Gly Ile
545                 550                 555                 560

Arg Ala Ile Glu Leu Leu Asp Glu Leu Ser Thr Leu Arg Leu Val Asp
                565                 570                 575

Lys Glu Asn Arg Tyr Glu Pro Leu Thr Ser Glu Val Glu Lys Glu Phe
            580                 585                 590

Ile Asp Leu Asp Ala Leu Tyr Glu Arg Ile Arg Ala Glu Arg Asp Ala
        595                 600                 605

Leu Gln Asp Val His Arg Ala Ile Cys Asp His Asn Glu Tyr Leu Gln
    610                 615                 620

Thr Gln Leu Gln Ile Tyr Gly Ser Tyr Leu Asn Asn Ala Arg Ser Gln
625                 630                 635                 640

Ile Lys Pro Ser His Ser Asp Ser Lys Gly Phe Ser Arg Gly Val Gly
                645                 650                 655

Val Val Gly Ile Lys Pro Lys Asn Ile Lys Ser Ser Asn Thr Val Lys
            660                 665                 670
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Leu Ser Ser Gln Gln Leu Lys Lys Glu Ser Val Leu Leu Asn Cys Thr
675 680 685

Ile Pro Glu Phe Asn Val Ser Asn Thr Tyr Phe Thr Phe Ser Ser Pro
690 695 700

Ser Thr Asp Asn Phe Val Ile Ala Val Tyr Gln Arg Gly His Ser Lys
705 710 715 720

Val Leu Val Glu Val Cys Ile Cys Leu Asp Asp Val Leu Gln Arg Arg
725 730 735

Tyr Ala Ser Asn Pro Val Val Asp Leu Gly Phe Leu Thr Phe Glu Ala
740 745 750

Asn Lys Leu Tyr His Leu Phe Glu Gln Leu Phe Leu Arg Lys
755 760 765

WHAT IS CLAIMED IS:

1. A method of blocking a Ras-induced effect on a cell, comprising a step of introducing a GTPase Activating (GAP) protein to said cell.

2. A method of Claim 1, wherein said Ras is an oncogenic Ras.

3. A method of Claim 1, wherein said Ras substantially lacks GTPase activity.

4. A method of Claim 1, wherein said effect is induction of cell proliferation or transformation.

5. A method of treating an oncogenic Ras transformed cell comprising the step of introducing to said cell a GAP protein capable of suppressing the transformation of said cell.

6. The method of either Claim 1 or 5, wherein said cell is a eukaryotic cell, including a mammalian cell, including a human cell.

7. The method of either Claim 1 or 5, wherein said step of introducing is by expression of a nucleic acid encoding said GAP protein.

8. A method for the manufacture of a pharmaceutical composition for treating an oncogenic Ras transformed cell comprising admixing a GAP protein capable of suppressing the transformation of said cell with a pharmaceutically acceptable carrier.

9. The method of any of Claims 1,5or 8 wherein said GAP protein binds to said Ras protein with a Kd of less than 200 nM.

10. The method of any of Claims 1,5 or 8 wherein said GAP protein is selected from the group of:

a) a fragment of a mammalian GAP protein;

b) a fragment of a mammalian NF1-GRD protein;

c) a homologue or mimetic of a or b; and

d) the proteins defined by SEQ ID NO: 1 or SEQ ID NO: 2.

11. The method of any of Claims 1, 5 or 8 wherein said GAP protein is selected from the group of:

a) a fragment of a mammalian GAP protein having a wild type sequence, including a human GAP protein; and

b) a fragment of a mutant mammalian GAP protein having a sequence with an amino acid substitution at a position corresponding to a position 1063 through 1651 of NF1 or the corresponding region of other GAP proteins.

12. A method of Claim 11, wherein said substitution is a conservative substitution.

13. The method of any of Claims 1, 5 or 8 wherein said GAP protein interacts with Ras and blocks interaction of an effector molecule which binds to Ras at a position from 32 to 40 or from 59 to 65.

14. A method of Claim 2, wherein said GAP protein does not block signal transduction of non-oncogenic Ras.

15. A method of either of Claim 1 or 2, further comprising the steps of identifying the responsible oncogenic Ras and selecting said GAP protein which blocks said identified oncogenic Ras.

16. A method of identifying appropriate GAP proteins useful for treating a mutated Ras-induced condition of a eukaryote cell comprising the steps of:

a) identifying the mutated Ras which induces said condition; and

b) screening various GAP variants for specific variants which are capable of blocking said condition.

17. A method of Claim 16, wherein said eukaryote cell is a mammalian cell, including a human cell.

18. A method of 16, further comprising additional screening to determine which GAP variants have minimal effect on non-mutated Ras effects.

19. A GAP protein capable of blocking transformation of a cell, where said transformation is due to an oncogenic Ras.

20. A protein of Claim 19, wherein said GAP is selected from the group of:

a) a fragment of a mammalian GAP protein;

b) a fragment of a mammalian NF1-GRD protein;

c) a homologue or mimetic of a or b; and

d) a protein defined by SEQ ID NO: 1 or SEQ ID NO: 2.

21. A protein of Claim 19, selected from the group of:

a) a fragment of a mammalian GAP protein having a wild type sequence, including a human GAP protein; and

b) a fragment of a mutant mammalian GAP protein having a sequence with an amino acid substitution at a position corresponding to a position from 1063 through 1651 or the corresponding region of other GAP proteins.

22. A protein of Claim 21, wherein said substitution is a conservative substitution.

23. A protein of Claim 19, wherein said protein interacts with Ras and blocks interaction of an effector molecule which binds to Ras at a position from 32 to 40 or from 59 to 65.

24. A protein of Claim 19, wherein said cell is a eukaryotic cell, including a mammalian cell, including a human cell.

25. A protein of Claim 19, wherein said oncogenic Ras substantially lacks GTPase activity.

26. A protein of Claim 19, which binds to said Ras protein with a Kd of less than 200 nM.

27. A protein of Claim 19, wherein said protein interferes with interaction of Ras•GTP with an effector compound.

28. An isolated nucleic acid encoding a protein normally expressed as a protein of Claim 19.

29. A pharmaceutical composition for treating an oncogenic Ras transformed cell comprising a GAP protein capable of suppressing the transformation of said cell and a pharmaceutically carrier.

30. The pharmaceutical composition of claim 29 wherein the GAP protein binds to said Ras protein with a Kd of less than 200 nM.

31. The pharmaceutical composition of claim 29 wherein said GAP protein is selected from the group of:

a) a fragment of a mammalian GAP protein;

b) a fragment of a mammalian NF1-GRD protein;
c) a homologue or mimetic of a or b; and
d) the proteins defined by SEQ ID NO: 1 or SEQ ID NO: 2.

32. The pharmaceutical composition of claim 29 wherein said GAP protein is selected from the group of:
a) a fragment of a mammalian GAP protein having a wild type sequence, including a human GAP protein; and
b) a fragment of a mutant mammalian GAP protein having a sequence with an amino acid substitution at a position corresponding to a position 1063 through 1651 of NF1 or the corresponding region of other GAP proteins.

33. The pharmaceutical composition of claim 29 wherein said GAP protein interacts with Ras and blocks interaction of an effector molecule which binds to Ras at a position from 32 to 40 or from 59 to 65.

34. The use of a GAP protein capable of suppressing the transformation of an oncogenic Ras transformed cell and a pharmaceutically carrier for treating said oncogenic Ras transformed cell.

35. The use of a GAP protein capable of suppressing the transformation of an oncogenic Ras transformed cell for the manufacture of a medicament for treating said oncogenic Ras transformed cell.

36. The use of either Claim 34 or 35 in which the GAP protein binds to said Ras protein with a Kd of less than 200 nM.

37. The use of either Claim 34 or 35 in which the GAP protein is selected from the group of:

a) a fragment of a mammalian GAP protein;
b) a fragment of a mammalian NF1-GRD protein;
c) a homologue or mimetic of a or b; and
d) the proteins defined by SEQ ID NO: 1 or SEQ ID NO: 2.

38. The use of either Claim 34 or 35 in which the GAP protein is selected from the group of:
a) a fragment of a mammalian GAP protein having a wild type sequence, including a human GAP protein; and
b) a fragment of a mutant mammalian GAP protein having a sequence with an amino acid substitution at a position corresponding to a position 1063 through 1651 of NF1 or the corresponding region of other GAP proteins.

39. The use of either Claim 34 or 35 in which the GAP protein interacts with Ras and blocks interaction of an effector molecule which binds to Ras at a position from 32 to 40 or from 59 to 65.

FIGURE 1A

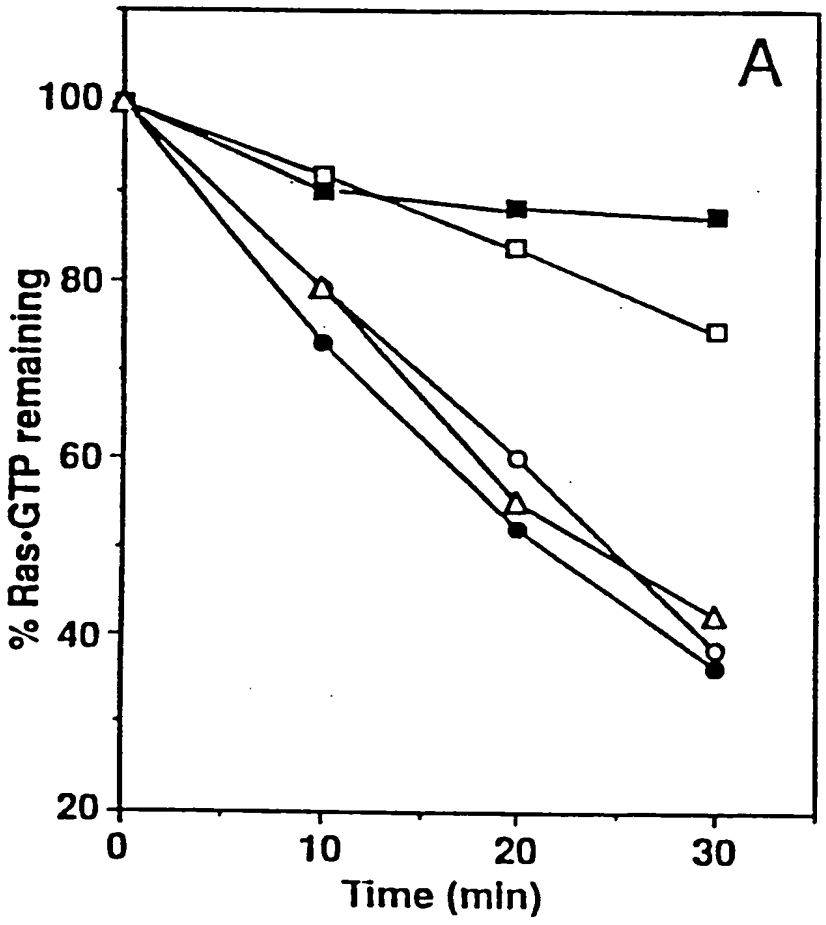

FIGURE 1B